

MINUTES OF THE 49th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in the Pi i Sunyer Hall, Fira de Barcelona, Barcelona, Spain on Thursday 26 September, 2013 at 18:30

Present:	Dr. Andrew J.M. Boulton	(President)
	Dr. Stefano Del Prato	(Vice President)
	Dr. Bernard Thorens	(Vice President)
	Dr. Michael Roden	(Honorary Treasurer)
	Dr. Mark Walker	(Honorary Secretary)
	Dr. Cees J. Tack	(Chair, PGEC)
	Dr. Viktor Jörgens	(Executive Director)
	Dr. Monika Grüsser	(Vice Director)
	and 51 members	

The President, Dr. Boulton, welcomed everyone to the 49th General Assembly. He asked those present to stand in memory of the following members, who had passed away: Drs. Georg Eisenbarth, John Hutton, Harry Keen, Carol Lurie, Irina G. Obrosova, Samuel Rahbar, Jean-Louis Richard, Richard Rubin and Patrick Vexiau.

1. MINUTES 48th GENERAL ASSEMBLY 2012

Since there were no comments, the minutes were unanimously approved and officially signed as a correct record.

2. REPORTS

a) President

The President's report to the members on the activities of EASD was given in the President's Address before the Minkowski Lecture. It is available under: <http://www.easdvirtualmeeting.org/resources/6926>

The President announced the Claude Bernard Lecturer 2014: Dr. Domenico Accili.

The President announced the Medical Devices in Diabetology meeting on 26/27 February 2014. He said better post-marketing surveillance was required and the Swedish project of regulating pumps would be looked at.

The President expressed his thanks to all partners. Dr. Boulton reported that as expected the EASD Annual Meeting in Barcelona was doing very well and the number of delegates attending had slightly increased. Dr. Boulton

thanked all members of the EASD Office and the Executive Committee for their commitment and hard work.

b) Honorary Treasurer

i) Result of tax audit 2008-2010/actions to be taken
In autumn 2012 for the second time, a control of the EASD by the Inland Revenue took place. These controls will always occur every three years due to the large turnover of the Association. In August 2013, EASD received the draft conclusion of this tax control. The non-profit status of the EASD is beyond all question; the main issue is the question of the taxation of the income from industry exhibition and symposia. EASD itself does not handle the industry exhibition and the symposia organized by third parties; these activities are taken care of by a professional congress organizer which is actually Interplan in Munich. In common with other medical associations in Germany, EASD has a contract, in our case with Interplan, giving them permission to organize these activities on their own responsibility without interfering with EASD concerning the organization and the fund raising. The basis of this collaboration is a loan contract and the income from such a loan contract is considered to be tax free. This legal and taxation construction is the basis of a healthy financial situation of German academic medical societies. The Düsseldorf tax authorities questioned this regulation and even asked their superiors for comments. In August 2013, they came up with the opinion that all incomes of EASD from these loan contracts, starting with 2008, are considered by the Inland Revenue as a taxable income. Taking into account corporation and local business tax, the result is that a total of Euro 5.4 million will likely have to be paid at the beginning of 2014. Following the advice of legal and

taxation advisors, EASD will oppose this decision and will eventually go to court. Nevertheless, this amount will be paid in advance to avoid the potential addition of interest on this sum should we elect to await the outcome of our appeal and should this be negative.

As we allowed for this potential outcome last year, no transfers have been carried forward from EASD to EFSD resulting in a reserve of Euro 5.2 million being kept on the accounts which is more than usual. Now EASD, following the income from the last Annual Meetings, will be able to cover the taxation imposed, but the Association will nevertheless have a hard time to finance its activities in the first six months of 2014 until a substantial income from the Meeting in Vienna has occurred.

The final decision regarding these matters will be made by the Düsseldorf Inland Revenue and their report could reach EASD at the beginning of 2014.

Following the advice from a specialist lawyer in Bonn, it was unanimously decided that when requested, the current tax bill of Euro 5.4 million should be paid; after that, we will challenge the decision in court. It was also unanimously decided to seek further advice from the lawyer concerning the way forward.

Dr. Roden explained that with the possibility of this tax invoice in mind, EASD had made no financial transfer to EFSD in 2012, resulting in EASD having enough funds to pay the tax bill. However, this will necessitate making savings in the first half of 2014 until an income has been received from the Annual Meeting in Vienna. Such savings could include reducing the costs of the Annual Meeting by no longer printing a Provisional Programme and no longer funding a Welcome and Farewell party. The decision had already been made to increase registration fees moderately.

Dr. Boulton thanked the Honorary Treasurer for his report and asked if there were any questions. Dr. Lenzen asked if there were any plans to relocate EASD. Dr. Boulton said that there were no relocation plans at that time.

c) Honorary Auditors

The President asked the Honorary Auditor, Dr. Peter Diem, for his report. Dr. Diem confirmed that the accounts had been checked carefully by Dr. Luis Gardete-Correia and were in perfect order. Dr. Boulton asked for the vote to accept the accounts.

The Honorary Treasurer was unanimously discharged (35 votes in favour and 6 abstentions).

d) Honorary Secretary

The Honorary Secretary reported that 2321 abstracts had been received for the Annual Meeting in Barcelona. Of these, 1360 were accepted: 264 oral presentations and 1096 poster presentations. The main countries submitting abstracts are the United States, the United Kingdom, Japan and Germany. The palm questionnaire rating the 48th Annual meeting in Berlin gave excellent feedback on the venue facilities, the scientific programme, the Prize Lectures, the EASD symposia, the oral presentations and abstracts, the poster presentations, the Industry symposia and the Associations' Village. The Rising Star Symposium continues to identify promising and innovative young researchers in Europe.

The Honorary Secretary concluded by reporting that the Programme Committee for 2014 had been appointed and had had its first meeting during the Annual Meeting in Barcelona. It would then meet in May 2014 to select the abstracts on an anonymous basis for the Annual Meeting in 2014.

Dr. Walker closed his report by thanking all those who had forwarded ideas for the scientific programme and all members of the EASD staff, in particular Mrs. H. Goliberzuch and Mrs. M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Boulton thanked Dr. Walker for his diligence and asked if there were any questions. There were no further comments.

e) Editor-in-Chief, Diabetologia

In the absence of Dr. Juleen Zierath, Editor-in-Chief, Diabetologia, the President reported the following information:

The Committee on Publication Ethics had put together ethical guidelines for peer-reviewers. From July 2013, all papers returned for revision underwent plagiarism detection. Springer is as yet unable to check images for possible manipulation, but are aware such a service is of high priority for the journal. The Scientific Integrity Panel (SIP) set up a policy outlining what is expected of authors and how to deal with cases of misconduct which was published on the Diabetologia website in April 2013. Mr. Tony Kirby, a former press officer with the Lancet, has helped to publicise papers by issuing press releases.

In total, 1,990 articles were submitted to Diabetologia in 2012 and 17% were published. The proportion of papers triaged increased to 63% in the first half of 2013. On average, papers are triaged in 3 days and peer-reviewed in 30 days.

Dr. Boulton expressed his thanks to Dr. Zierath in her absence for her co-operation and hard work. Thanks were also expressed to the team in Bristol, especially to Dr. Judy Naylor, and the outgoing associate editors.

f) Chair, Postgraduate Education Committee

Dr. Tack reported on the courses that had taken place in 2012 and 2013: Almaty/Kazakhstan, Lviv/Ukraine and Kazan/Russia. Dr. Tack said all courses in 2012 and 2013 had been very successful and had attracted delegates from many of the countries neighbouring the one where the course was held. Dr. Tack said a course was being planned in Budapest/Hungary in November 2014 and additional courses will be agreed upon with the incoming PGEC Chair.

Dr. Boulton thanked Dr. Tack for his co-operation and commitment to postgraduate education.

g) Chair, Extra-European Postgraduate Activities

Dr. Czupryniak reported that a course had successfully been organised in Dubai/United Arab Emirates in 2012. In August 2013, a course was held in India when 4 cities (Chennai, Ahmedabad, Lucknow and Chandigarh) were visited. The main Extra-European course in collaboration with IDF and ADA in 2013 will take place in Sri Lanka in November. Also in November, another course will be held in the Gulf Region (Oman).

Dr. Czupryniak brought his report to an end by thanking Dr. Boulton for his support and the EASD team for their help in organising the courses.

Dr. Boulton thanked Dr. Czupryniak for his co-operation and hard work.

3. ELECTIONS

Honorary Secretary (2013–2016)

The election of Dr. Per-Henrik Groop was unanimously approved with 40 votes.

Chair, PGEC (2013–2016)

The election of Dr. Leszek Czupryniak was unanimously approved with 41 votes and 1 abstention.

Council Members (2014 – 2017)

The election of Drs. Bo Ahrén, Sehnaz Karadeniz, Michele Solimena, Nicholas Tentolouris was unanimously approved with 37 votes each and 4 abstentions.

Editor-in-Chief, Diabetologia (2013–2015) extension of office

The election of extension of office of Dr. Juleen Zierath was unanimously approved with 41 votes.

4. STUDY GROUPS

i) Non-Alcoholic Fatty Liver Disease (NAFLD) Study Group

The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.

ii) EASD Diabetes and Cancer Study Group

The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.

iii) Gluco-Incretin Biology and Clinical Applications Study Group

The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.

iv) Metabolic Imaging Study Group

The General Assembly was informed of the decision by the Executive Committee and Council to endorse the initiation of this EASD Study Group.

5. HONORARY MEMBERSHIP

The nomination of Drs. Anthony Cerami, Willy Malaisse, John D. Ward was unanimously approved. Dr. Boulton thanked them for their outstanding contribution to diabetes research.

6. ANY OTHER BUSINESS

Dr. Boulton reported that from January 2014 only an electronic version of the journal would be available for members. He added that this was the correct way forward both economically and ecologically. He said this would not affect re-prints which would continue to be available. An email will be sent to all members informing them of this situation and a monthly email will be sent with the table of contents for each month's journal.

The President thanked Dr. Walker, retiring Honorary Secretary, and Dr. Cees Tack, retiring Chair of the PGEC, for their friendly collaboration and diligence during their term of office. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 49th EASD Annual Meeting. He warmly thanked Dr. Jörgens, Dr. Grüsser and the EASD team in Düsseldorf for their dedicated work.

Dr. Boulton brought the General Assembly to a close at 19:25.

Agenda for the 50th General Assembly of the European Association for the Study of Diabetes

to be held in the Randle Hall at Messe Wien Exhibition & Congress Center, Vienna, Austria
on Tuesday, 16 September 2014 at 18:30

1. Minutes of the 49th General Assembly, Barcelona, Spain 2013

2. Reports

a) President	Andrew J.M. Boulton
i) Membership fees 2016	
ii) Retirement Executive Director	
b) Honorary Treasurer	Michael Roden
c) Honorary Auditors	Peter Diem
	Luis M. Gardete-Correia
d) Honorary Secretary	Per-Henrik Groop
e) Editor-in-Chief, Diabetologia	Juleen Zierath
f) Chair, Postgraduate Education Committee	Leszek Czupryniak

3. POSITIONS

To be elected for retiring member(s):

3.1 Elections

a) President Extension of office (2014-2015)	Andrew JM Boulton
b) President (2015-2018)	Andrew JM Boulton
c) Vice President (2014-2017)	Stefano Del Prato
d) Honorary Treasurer Extension of office (2014-2016)	Michael Roden
d) Honorary Auditors (2014-2017)	Peter Diem and Luis M. Gardete-Correia
e) Council Members (2015-2018)	Henning Beck-Nielsen
	Svitlana Bolgarskaya
	Anna Novials
	Raimund Weitgasser

4. Study Groups

- a) Reactive Metabolites in Diabetes Study Group

5. Honorary Membership

6. Any other business

50th EASD Annual Meeting of the European Association for the Study of Diabetes

Vienna, Austria, 15 – 19 September 2014

Abstracts

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- OP 03 Evolving tools in education
- OP 04 Brown adipose tissue
- OP 05 Factors driving islet cell development
- OP 06 Novel mechanism of glucose tolerance
- OP 07 GLP-1 analogues: clinical efficacy
- OP 08 Matters of the heart
- OP 09 Diabetic retinopathy
- OP 10 Entero-pancreatic endocrinology
- OP 11 Lifestyle factors and prediction of type 2 diabetes
- OP 12 The many faces of advanced glycation
- OP 13 Clinical studies with GLP-1 analogues
- OP 14 Weight regulation and obesity
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- OP 17 Intra- and inter-islet cell signalling
- OP 18 Novel genes for type 2 diabetes and its complications
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- OP 27 Protecting the periphery
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- OP 48 Novel targets for anti-inflammatory therapies

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- PS 002 Aetiological epidemiological studies of type 2 diabetes
- PS 003 Type 1 diabetes: epidemiology
- PS 004 Epidemiology of obesity and ectopic fat
- PS 005 Descriptive epidemiology of diabetes
- PS 006 Epidemiology of diabetes: comorbidities and complications
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- PS 008 Type 1 diabetes: genes and biomarkers
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- PS 014 Beta cell proliferation and differentiation
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- PS 017 Transgenic animal models of type 1 and type 2 diabetes
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| PS 039 Glucagon | PS 091 Tailored diabetes care |
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OP 01 SGLT2 inhibitors: new outcome studies

1

Fixed dose combinations of empagliflozin/linagliptin for 52 weeks as add-on to metformin in subjects with type 2 diabetes

S. Patel¹, R. DeFronzo², A. Lewin³, D. Liu⁴, R. Kaste⁴, H.J. Woerle⁵, U.C. Broedl⁵;

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Background and aims: A randomized, double-blind, parallel group Phase III study evaluated the efficacy and safety of fixed dose combinations (FDCs) of empagliflozin/linagliptin (EMPA/LINA) as add-on to metformin in subjects with type 2 diabetes (T2DM).

Materials and methods: Subjects were randomized to EMPA 25 mg/LINA 5 mg (n=137), EMPA 10 mg/LINA 5 mg (n=136), EMPA 25 mg (n=141), EMPA 10 mg (n=140), or LINA 5 mg (n=132) as add-on to stable-dose metformin for 52 weeks. Primary analysis was at week 24. Exploratory endpoints at week 52 were changes from baseline in HbA1c, body weight, systolic and diastolic blood pressure (SBP and DBP), and percentage of subjects with baseline HbA1c $\geq 7\%$ who reached HbA1c $< 7\%$. Efficacy was evaluated in 674 subjects (mean [SD] age 56.2 [10.2] years; weight 86.2 [18.7] kg; BMI 31.0 [5.5] kg/m²; HbA1c 7.98 [0.85] %).

Results: Compared with LINA 5 mg and their respective EMPA monotherapies, both EMPA/LINA FDCs led to significant reductions in HbA1c and higher percentages of subjects with HbA1c $< 7\%$ at week 52 (Table). Compared with LINA 5 mg, body weight was reduced with EMPA 25 mg/LINA 5 mg (difference: -3.2 kg [95% CI 4.2, 2.2]; $p < 0.001$) and EMPA 10 mg/LINA 5 mg (difference: 2.8 kg [95% CI 3.8, 1.7]; $p < 0.05$). The FDCs did not reduce body weight compared with their respective EMPA monotherapies. Changes from baseline in SBP and DBP were 0.3 mmHg and -0.6 mmHg, respectively, with LINA 5 mg. EMPA and FDCs reduced SBP from baseline by 2.8 to 3.6 mmHg and DBP by 1.8 to 2.2 mmHg. Adverse events (AEs) were reported in 71.5%, 69.1%, 73.0%, 68.6% and 68.9% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively, over 52 weeks. Confirmed hypoglycaemic AEs (glucose ≤ 70 mg/dL and/or requiring assistance) were reported in 3.6%, 2.2%, 3.5%, 1.4% and 2.3% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively; none required assistance. AEs consistent with urinary tract infection were reported in 10.2%, 9.6%, 13.5%, 11.4% and 15.2% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively, and AEs consistent with genital infection were reported in 2.2%, 5.9%, 8.5%, 7.9% and 2.3% of subjects in these groups, respectively.

Conclusion: As add-on to stable-dose metformin in subjects with T2DM, FDCs of EMPA 25 mg/LINA 5 mg and EMPA 10 mg/LINA 5 mg for 52 weeks significantly reduced HbA1c compared with LINA 5 mg and compared with the respective EMPA monotherapies. The FDCs were well tolerated, with overall safety profiles similar to those known for the individual components.

	EMPA 25 mg/ LINA 5 mg (n=133)	EMPA 10 mg/ LINA 5 mg (n=135)	EMPA 25 mg (n=139)	EMPA 10 mg (n=137)	LINA 5 mg (n=128)
Baseline HbA1c (%)	7.90 (0.07)	7.95 (0.07)	8.01 (0.07)	8.00 (0.08)	8.02 (0.08)
Change from baseline in HbA1c (%) at week 52	-1.21 (0.08)	-1.04 (0.07)	-0.69 (0.07)	-0.70 (0.08)	-0.45 (0.08)
Difference vs EMPA 25 mg (95% CI)	-0.52 (-0.73, -0.31)***	—	—	—	—
Difference vs EMPA 10 mg (95% CI)	—	-0.34 (-0.55, -0.14)**	—	—	—
Difference vs LINA 5 mg (95% CI)	-0.77 (-0.98, -0.55)***	-0.60 (-0.81, -0.38)***	—	—	—
Subjects with HbA1c $\geq 7\%$ at baseline* who had HbA1c $< 7\%$ at week 24, n (%)	59 (48.0)	66 (51.6)	43 (32.6)	40 (32.0)	34 (28.6)
Odds ratio vs EMPA 25 mg (95% CI)	1.96 (1.13, 3.42)*	—	—	—	—
Odds ratio vs EMPA 10 mg (95% CI)	—	2.36 (1.35, 4.13)**	—	—	—
Odds ratio vs LINA 5 mg (95% CI)	2.46 (1.38, 4.39)**	2.92 (1.65, 5.17)***	—	—	—

Baseline values are mean (SE). Changes are adjusted mean (SE) based on mixed model repeated measures approach using observed cases in subjects treated with ≥ 1 dose of trial medication who had a baseline and on-treatment HbA1c value. n=123 for EMPA 25 mg/LINA 5 mg, n=128 for EMPA 10 mg/LINA 5 mg, n=132 for EMPA 25 mg, n=125 for EMPA 10 mg, n=119 for LINA 5 mg. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Clinical Trial Registration Number: NCT01422876

Supported by: Boehringer Ingelheim and Eli Lilly

2

Empagliflozin compared with glimepiride as add-on to metformin for 2 years in patients with type 2 diabetes

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¹Steno Diabetes Center, Gentofte, Denmark, ²Boehringer Ingelheim Norway KS, Asker, Norway, ³Boehringer Ingelheim, Biberach, ⁴Boehringer Ingelheim, Ingelheim, Germany.

Background and aims: We performed a Phase III trial, EMPA-REG H2H-SUTM, to compare the efficacy and safety of empagliflozin (EMPA) and glimepiride (GLIM) as add-on to metformin (MET) in patients with type 2 diabetes (T2DM).

Materials and methods: Patients were randomized and treated double-blind with EMPA 25 mg (n=765) or GLIM 1-4 mg (n=780) for 104 weeks (mean [SD] age 55.9 [10.4] years; BMI 30.1 [5.3] kg/m²). The primary endpoint was change from baseline in HbA1c. Key secondary endpoints were change from baseline in weight, occurrence of confirmed hypoglycaemic adverse events (AEs; plasma glucose ≤ 70 mg/dL and/or requiring assistance) and changes from baseline in systolic and diastolic blood pressure (SBP and DBP).

Results: At week 104, EMPA significantly reduced HbA1c versus GLIM (Table). Confirmed hypoglycaemic AEs were reported in 2.5% of patients on EMPA and 24.2% on GLIM (adjusted risk ratio: 0.102 [95% CI 0.065, 0.162]; $p < 0.001$); no patients on EMPA and 2 on GLIM required assistance. EMPA significantly reduced weight and SBP versus GLIM (Table). At week 104, adjusted mean (SE) change from baseline in DBP was 1.8 (0.3) mmHg with EMPA compared with 0.9 (0.3) mmHg with GLIM (difference versus GLIM: -2.7 mmHg [95% CI 3.4, 1.9]; $p < 0.001$). AEs were reported in 86.4% and 86.3% of patients on EMPA and GLIM, respectively. AEs consistent with urinary tract infection were reported in 13.7% and 13.1% of patients, and AEs consistent with genital infection were reported in 11.8% and 2.2% of patients, on EMPA and GLIM, respectively.

Conclusion: This large and long-term trial showed that EMPA 25 mg for 104 weeks as add-on to MET was well tolerated and led to a greater HbA1c

reduction, weight loss and BP reduction with a low risk of hypoglycaemia compared with GLIM.

	Glimepiride 1–4 mg	Empagliflozin 25 mg
Baseline HbA _{1c} , %	7.92 (0.03)	7.92 (0.03)
Change from baseline in HbA _{1c} at week 104, %	-0.55 (0.03)	-0.66 (0.03)
Difference in change from baseline in HbA _{1c} vs. glimepiride, % (95% CI)		-0.11 (-0.19, -0.02)*
Baseline body weight, kg	83.0 (0.7)	82.5 (0.7)
Change from baseline in body weight at week 104, kg	1.3 (0.1)	-3.1 (0.1)
Difference in change from baseline in body weight vs. glimepiride, kg (95% CI)		-4.5 (-4.8, -4.1)***
Baseline SBP, mmHg	133.5 (0.6)	133.4 (0.6)
Change from baseline in SBP at week 104, mmHg	2.5 (0.4)	-3.1 (0.4)
Difference in change from baseline in SBP vs. glimepiride, mmHg (95% CI)		-5.6 (-6.8, -4.4)***
Baseline values are mean (SE). Changes from baseline are adjusted mean (SE) based on ANCOVA in full analysis set with last observation carried forward imputation; values after anti-diabetic rescue medication were excluded from analysis of HbA _{1c} , body weight, blood pressure; values after a change in anti-hypertensive medication were excluded from blood pressure analyses. *p<0.025 vs GLIM; ***p<0.001 vs GLIM.		

Clinical Trial Registration Number: NCT01167881

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Energy balance following sodium-glucose co-transporter-2 (SGLT2) inhibition

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Background and aims: SGLT2 inhibitors lower glycaemia by inducing urinary glucose excretion (UGE), with the attendant calorie loss. Evidence suggests that the resulting weight loss (WL) is less than expected from UGE.

Materials and methods: To quantify this phenomenon we analysed data from 86 type 2 diabetic (T2D) patients (39 women, age = 58 ± 9 years, BMI = 29.8 ± 4.5 kg/m², HbA_{1c} = 7.8 ± 0.8%, FPG = 169 ± 41 mg/dL, eGFR = 89 ± 19 mL/min^{1.73}m², μ ± SD), the per-protocol completers cohort of a clinical trial who received empagliflozin (25 mg/day) for 90 weeks with frequent (n=11) assessments of body weight, eGFR, and FPG. Time-dependent glucose filtration was calculated as the product of eGFR and FPG, time-dependent UGE was estimated by assuming - from previous direct measurements - a quasi-linear relationship between fractional UGE and glycaemia. The relation of calorie-to-weight changes was estimated using a mathematical model (<http://bwsimulator.niddk.nih.gov>) that simulates the time-course of WL for a given change in calorie balance.

Results: At week 90, WL averaged -3.2 ± 4.2 kg (range -17.0 to +5.5); over 90 weeks, UGE averaged 54 ± 15 g/day (fractional UGE = 45 ± 4%). The observed WL corresponded to a calorie deficit of -78 ± 103 kcal/day. On the other hand, the observed calorie loss (-217 ± 59 kcal/day) predicted a WL of -8.7 ± 2.4 kg (range -4.0 to -15.3 kg) over 90 weeks. Thus, patients lost only 38 ± 53% of the WL predicted by their glycosuria. As previous studies showed that empagliflozin does not affect either resting or meal-induced energy expenditure, patients likely increased their energy intake (by an estimated +138 ± 116 kcal/day). This excess calorie intake was inversely related to baseline BMI (partial r = -0.33, p<0.01) and positively to baseline eGFR (partial r = 0.30, p<0.01).

Conclusion: Chronic glycosuria elicits an adaptive increase in energy intake, particularly in leaner patients with preserved renal function. Combining SGLT2 inhibition with strategies to maintain energy intake or curb appetite is expected to be associated with major WL.

Clinical Trial Registration Number: NCT00881530

Supported by: Boehringer Ingelheim and Eli Lilly

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Randomised, double-blind trial of dual add-on saxagliptin plus dapagliflozin vs saxagliptin or dapagliflozin add-on alone in poorly controlled type 2 diabetes on metformin

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Background and aims: SGLT2 and DPP-4 inhibitors have complementary mechanisms of action that can potentially improve glucose control with weight loss and a low risk of hypoglycaemia. We compared the efficacy and safety of dual add-on of saxagliptin (SAXA) and dapagliflozin (DAPA) with SAXA and DAPA alone in patients with type 2 diabetes mellitus (T2DM) poorly controlled with metformin.

Materials and methods: In this 24-week, multicenter, randomized, double-blind, active-controlled trial, adults with T2DM and A1C ≥8.0% and ≤12.0%, received SAXA 5 mg and DAPA 10 mg once daily compared with SAXA and placebo (PBO) or DAPA and PBO on background of metformin XR ≥1500 mg/d. The primary end point was the adjusted mean change in A1C from baseline to week 24. Safety and tolerability assessments included adverse events (AEs) and hypoglycaemia.

Results: A total of 534 patients were randomized. Mean ± SD A1C at baseline in SAXA+DAPA, SAXA+PBO, and DAPA+PBO groups was 8.9 ± 1.2%, 9.0 ± 1.1%, and 8.9 ± 1.2%, respectively. Adjusted mean reduction from baseline in A1C was -1.47% in SAXA+DAPA compared with -0.88% in SAXA+PBO (difference -0.59%; 95% CI [-0.81, -0.37]; P<0.0001) and -1.20% in DAPA+PBO (difference -0.27%; 95% CI [-0.48, -0.05]; P<0.02). The adjusted mean proportion of patients achieving A1C <7% was 41% in SAXA+DAPA compared with 18% in SAXA+PBO (difference of 23%; 95% CI [15, 32]) and 22% in DAPA+PBO (difference of 19%; 95% CI [10, 28]). AEs occurred in 48.6%, 52.8%, and 48.6% of patients in the SAXA+DAPA, SAXA+PBO, and DAPA+PBO groups, respectively. Urinary and genital infections occurred with the expected frequency previously reported. Incidence of hypoglycaemia was 1.1%, 0.6%, and 1.1%, respectively with no episodes of major hypoglycaemia.

Conclusion: This first report of triple therapy, adding a well-tolerated combination of DPP-4 and SGLT2 inhibitors to background metformin therapy in patients with T2DM poorly controlled with metformin, demonstrated that the dual add-on combination of SAXA and DAPA had greater improvements in glucose control than each component alone. More than 40% of poorly controlled T2DM patients receiving SAXA+DAPA achieved an A1C goal of <7%, with weight loss similar to DAPA alone and with very low hypoglycaemia risk.

Clinical Trial Registration Number: NCT01606007

Supported by: BMS and AstraZeneca

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Long-term efficacy and safety of canagliflozin in older patients with type 2 diabetes mellitus over 104 weeks

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Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor developed for the treatment of type 2 diabetes mellitus (T2DM), lowers plasma glucose by increasing urinary glucose excretion. This randomised, double-blind, Phase 3 study assessed the long-term efficacy and safety of CANA in older patients with T2DM over 104 weeks.

Materials and methods: Patients aged 55 to 80 years (N = 714) with T2DM inadequately controlled on a stable antihyperglycaemic agent (AHA) regimen received CANA 100 or 300 mg or placebo (PBO) during a 26-week core period followed by a 78-week extension (n = 624 [87%]). Efficacy endpoints were evaluated at 104 weeks and safety was assessed by adverse event (AE) reports.

Results: Mean baseline characteristics were similar across groups (age, 63.6 years; HbA_{1c}, 7.7%; fasting plasma glucose [FPG], 8.7 mmol/L; BMI, 31.6 kg/m²; estimated glomerular filtration rate [eGFR], 77.5 mL/min/1.73 m²). At 104 weeks, CANA 100 and 300 mg reduced HbA_{1c}, FPG, body weight, and systolic blood pressure (BP), with small increases in high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) compared with PBO (Table). Over 104 weeks, overall AE rates were 88.0%, 89.8%, and 86.1% with CANA 100 and 300 mg and PBO, respectively; AE-related

discontinuation rates were 4.6%, 10.2%, and 6.8%, and serious AE rates were similar across groups. CANA 100 and 300 mg were associated with a higher incidence than PBO of urinary tract infections (14.5%, 16.5%, 10.1%) and genital mycotic infections in females (23.9%, 18.7%, 4.3%) and males (5.6%, 10.9%, 1.4%). Incidences of osmotic diuresis-related AEs (eg, pollakiuria [increased urine frequency] and thirst; 9.1%, 12.3%, 5.5%) and AEs related to reduced intravascular volume (eg, postural dizziness and orthostatic hypotension; 5.4%, 5.9%, 1.7%) were higher with CANA 100 and 300 mg than PBO, but these AEs led to few discontinuations. The incidence of documented hypoglycaemia (≤ 3.9 mmol/L) was modestly higher with CANA 100 and 300 mg than PBO in patients on an AHA associated with hypoglycaemia (54.1%, 61.0%, 48.9%) as well as those on an AHA not associated with hypoglycaemia (18.3%, 10.9%, 6.6%); severe hypoglycaemia rates were low across groups.

Conclusion: CANA 100 and 300 mg improved glycaemic control, reduced body weight and BP, and were generally well tolerated in older patients with T2DM over 104 weeks, consistent with 26-week results; the AE profile was consistent with that seen in a broad range of CANA-treated patients.

Table. Summary of Efficacy Endpoints at Week 104 (mITT, LOCF)

Parameter	CANA 100 mg	CANA 300 mg	PBO
HbA _{1c} change, %	-0.32 (0.08)	-0.43 (0.08)	0.17 (0.08)
Difference vs PBO	-0.49 (-0.65, -0.32)	-0.60 (-0.77, -0.44)	
% of patients reaching HbA _{1c} <7.0% ^a	35.8	41.9	20.3
Difference vs PBO	15.6 (7.2, 24.0)	21.7 (13.0, 30.3)	
FPG change, mmol/L	-0.6 (0.2)	-0.7 (0.2)	0.6 (0.2)
Difference vs PBO	-1.2 (-1.6, -0.8)	-1.3 (-1.7, -0.9)	
Body weight % change	-3.0 (0.4)	-3.8 (0.4)	-0.6 (0.4)
Difference vs PBO	-2.3 (-3.1, -1.6)	-3.2 (-4.0, -2.4)	
Systolic BP change, mmHg	-1.2 (1.1)	-3.0 (1.1)	4.5 (1.1)
Difference vs PBO	-5.8 (-8.0, -3.5)	-7.5 (-9.8, -5.2)	
Triglycerides % change	3.5 (4.2)	11.7 (4.2)	7.9 (4.2)
Difference vs PBO	-4.4 (-13.5, 4.8)	3.9 (-5.4, 13.1)	
HDL-C % change	6.7 (1.5)	7.8 (1.5)	3.0 (1.5)
Difference vs PBO	3.6 (0.4, 6.9)	4.8 (1.5, 8.1)	
LDL-C % change	8.4 (2.9)	8.1 (3.0)	5.6 (3.0)
Difference vs PBO	2.8 (-3.7, 9.2)	2.5 (-4.0, 9.0)	
LDL-C/HDL-C % change	3.0 (3.1)	4.7 (3.2)	3.4 (3.2)
Difference vs PBO	-0.4 (-7.2, 6.4)	1.3 (-5.6, 8.2)	
Non-HDL-C % change	4.0 (2.3)	7.4 (2.4)	3.6 (2.4)
Difference vs PBO	0.4 (-4.7, 5.5)	3.8 (-1.4, 8.9)	

mITT, modified intent to treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; ANCOVA, analysis of covariance; CI, confidence interval. LS mean (SE) change from baseline using ANCOVA and PBO-subtracted LS mean (95% CI) for all parameters except for % of patients reaching HbA_{1c} <7.0%, % and PBO-subtracted % (95% CI) of patients.

Clinical Trial Registration Number: NCT01106651

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Safety of dapagliflozin in patients with type 2 diabetes mellitus and hypertension inadequately controlled by a renin-angiotensin system blocker with/without a second agent

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Background and aims: Hypertension is common in patients with type 2 diabetes mellitus (T2DM) and is often treated with an ACE inhibitor (ACEi) or an angiotensin receptor blocker (ARB) plus other antihypertensive (AHT) agents if needed. Dapagliflozin inhibits renal glucose reabsorption causing glucosuria, weight loss, diuresis and decreased BP. Dapagliflozin significantly reduced HbA_{1c} and seated and ambulatory systolic BP versus placebo in clinical studies of patients with T2DM and hypertension. Here we describe key safety data from these studies.

Materials and methods: In two, randomized, double-blind, 12-week studies, patients with T2DM and inadequate glycaemic control (HbA_{1c} 7.0–10.5%) and BP (seated systolic BP / diastolic BP: 140–164 / 85–104 mmHg) receiving an ACEi or an ARB (Study 1) plus a 2nd AHT drug (Study 2) were randomized to dapagliflozin 10 mg or placebo over 12 weeks. Preliminary efficacy results have been previously presented.

Results: In Study 1, 613 patients on an ACEi or ARB were randomized to treatment. In Study 2, 449 patients on an ACEi or ARB plus a 2nd AHT drug were randomized to treatment. In each study, similar proportions of dapagliflozin and placebo experienced adverse events (AEs), with few serious AEs reported. In Study 1 and 2, fewer dapagliflozin (1.0 and 0.4%, respectively) versus placebo (1.3 and 1.8%) patients withdrew due to an AE. Few hypoglycaemic events occurred (3.3 and 5.8% with dapagliflozin vs 1.3 and 2.7% with placebo), none of which led to discontinuation. No serious BP related safety issues were noted; one orthostatic hypotension AE occurred with dapagliflozin in Study 1. Serum uric acid decreased with dapagliflozin but there was no effect on serum sodium, potassium, or calcium, despite its diuretic effect (Table).

Conclusion: Dapagliflozin had a good safety profile over 12 weeks when used in combination with an ACEi or ARB ± 1AHT in patients with T2DM and inadequately controlled hypertension.

	ACEi/ARB (Study 1)	
	+ PBO (N = 311)	+ DAPA 10mg (N = 302)
At least 1 AE, N (%)	109 (35.0)	111 (36.8)
At least 1 SAE, N (%)	4 (1.3)	2 (0.7)
Calcium, mmol/L		
Baseline mean	2.37 (0.10)	2.37 (0.13)
Week 12 mean	2.37 (0.11)	2.38 (0.11)
Mean change	0.00 (-0.01, 0.01)	0.01 (-0.01, 0.03)
Potassium, mmol/L		
Baseline mean	4.48 (0.44)	4.44 (0.44)
Week 12 mean	4.52 (0.43)	4.47 (0.44)
Mean change	0.05 (0.00, 0.10)	0.03 (-0.02, 0.08)
Sodium, mmol/L		
Baseline mean	140.4 (2.94)	140.3 (2.94)
Week 12 mean	140.3 (3.02)	140.8 (3.03)
Mean change	-0.1 (-0.41, 0.21)	0.4 (0.05, 0.75)
Uric acid, µmol/L		
Baseline mean	318.24 (74.36)	321.22 (82.80)
Week 12 mean	317.06 (77.09)	299.21 (77.15)
Mean change	2.97 (-5.95, 11.30)	-16.06 (-24.98, -7.73)
	ACEi/ARB + 1 other AHT (Study 2)	
	+ PBO (N = 224)	+ DAPA 10mg (N = 225)
At least 1 AE, N (%)	93 (41.5)	98 (43.6)
At least 1 SAE, N (%)	2 (0.9)	6 (2.7)
Calcium, mmol/L		
Baseline mean	2.38 (0.10)	2.38 (0.13)
Week 12 mean	2.39 (0.11)	2.38 (0.11)
Mean change	0.01 (-0.00, 0.03)	-0.00 (-0.02, 0.02)
Potassium, mmol/L		
Baseline mean	4.36 (0.45)	4.41 (0.45)
Week 12 mean	4.44 (0.46)	4.42 (0.46)
Mean change	0.06 (0.01, 0.11)	0.00 (-0.06, 0.06)
Sodium, mmol/L		
Baseline mean	140.6 (2.96)	140.3 (2.81)
Week 12 mean	140.7 (3.11)	141.1 (2.96)
Mean change	0.0 (-0.41, 0.41)	0.8 (0.37, 1.23)
Uric acid, µmol/L		
Baseline mean	321.22 (78.22)	334.90 (94.94)
Week 12 mean	321.81 (77.39)	308.73 (90.36)
Mean change	-1.78 (-11.90, 8.92)	-25.58 (-35.69, -14.87)

N is the number of randomized patients who took ≥ 1 dose of double-blind medication. Includes data after rescue. Baseline and Week 12 means are shown with standard deviations and mean changes with 95% confidence intervals. ACEi, angiotensin converting enzyme inhibitor; AE, adverse event; AHT, antihypertensive drug; ARB, angiotensin receptor blocker; DAPA, dapagliflozin; PBO, placebo; SAE, serious adverse event
Calcium: 1 mmol/L = 4 mg/dL [Normal range: 2.13–2.55 mmol/L]
Potassium: 1 mmol/L = 1 mEq/L [Normal range: 3.7–5.2 mmol/L]
Sodium: 1 mmol/L = 1 mEq/L [Normal range: 135–145 mmol/L]
Uric acid: 1 µmol/L = 0.02 mg/dL [Normal range: 208.2–428.29 µmol/L]

Clinical Trial Registration Number: NCT01137474, NCT01195662

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OP 02 Nephropathy: biomarkers and infections

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Uric acid serum level affects serum level of cystatin C, independently of GFR, in patients with type 2 diabetes

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Background and aims: Cystatin C has been reported to be a reliable marker of GFR in both type 1 and type 2 diabetic patients with mild-to-moderate CKD (stages 2-3). Recently, elevated serum level of cystatin C has also been identified as a significant prognostic indicator for the development of cardiovascular disease in people with diabetes. However, there are limited data on factors, other than GFR, that influence its serum concentration, especially in adult patients with type 2 diabetes. The aim of our study was to identify such factors.

Materials and methods: In this cross-sectional study, 560 consecutive type 2 diabetic patients (252 men, 308 women), aged 65.0±10.0 years (mean±SD) were recruited from our outpatient diabetic clinic. All participants were Europeans. GFR was measured using 51Cr-EDTA (mGFR). Serum cystatin C was related to several clinical and biochemical parameters. Multivariate analysis was performed in order to identify factors independently associated with cystatin C serum level beyond mGFR. SPSS 18.0 was used for statistical analysis. A value of $p < 0.01$ was considered to indicate statistical significance.

Results: Cystatin C was significantly correlated with mGFR ($r = -0.590$; $p < 0.001$), age ($r = 0.365$; $p < 0.001$), ACR-albumin creatinine ratio ($r = 0.159$; $p = 0.001$), uric acid ($r = 0.299$; $p < 0.001$), albumin ($r = -0.206$; $p < 0.001$) and Hb ($r = -0.230$; $p < 0.001$). There were no significant relationships between serum cystatin C levels and other variables (diabetes duration, sex, glucose-lowering therapy, BMI, fasting glucose, HbA1c, liver function tests, lipid profile, thyroid function tests, white blood cells, electrolytes and hsCRP). However, in standard multiple regression analysis, only mGFR ($B = -0.008$; $p < 0.001$) and uric acid ($B = 0.040$; $p = 0.008$) were independent predictors of cystatin C level.

Conclusion: In adult patients with type 2 diabetes, uric acid serum level seems to affect cystatin C serum level independently of GFR. Hence, uric acid level should be considered when cystatin C based equations for GFR estimation are used.

Clinical Trial Registration Number: NCT01215994

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Associations of serum bicarbonate with renal and cardiovascular outcome in patients with diabetic nephropathy

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Background and aims: Low serum bicarbonate, one of the metabolic complications of impaired renal function, has been reported to be an independent predictor of renal function decline and mortality. To date, no study has investigated this association in patients with diabetic nephropathy (DN), whereas it has been suggested that acid-base balance is different in subjects with DM versus non-DM. We therefore investigated the association of serum bicarbonate with renal and cardiovascular (CV) outcome in specifically type 2 DM patients with DN.

Materials and methods: Post-hoc analysis of the RENAAL and IDNT trials, both randomised controlled trials (RCTs) investigating the effect of angiotensin receptor blockers on renal and CV outcomes in patients with DN due to type 2 DM. DN was defined as urinary albumin-to-creatinine ratio (UACR) ≥ 300 mg/g or proteinuria ≥ 900 mg/d. Serum bicarbonate was measured as total CO_2 . Linear regression analysis was used to examine baseline factors associated with serum bicarbonate concentration. In addition, Cox regression models were built to examine the longitudinal associations of serum bicarbonate with renal outcomes (incidence of end-stage renal disease (ESRD), the

combined endpoint ESRD or doubling of serum creatinine (DSCR)) and CV outcome (incidence of fatal/non-fatal stroke/myocardial infarction). Serum bicarbonate concentration was studied as continuous variable and stratified in quartiles to allow assessment of non-linear associations.

Results: We included 2628 patients (mean age 60 ± 7 (SD) years, 65% male, 61% white) with mean estimated GFR (eGFR) 44 ± 16 mL/min/1.73m² and median UACR of 1366 mg/g (interquartile range 682 - 2653). Mean serum bicarbonate level was 24.3 ± 3.7 mEq/L and 602 patients had serum bicarbonate levels < 21 mEq/L. Multivariate linear regression analyses showed significant associations of serum bicarbonate with age, diuretic use, total cholesterol, potassium, chloride, phosphate and albuminuria (all $p < 0.05$), and particularly with eGFR ($p < 0.001$). During follow-up for 2.8 ± 1.0 years, 948 (36%) patients developed ESRD, 731 (28%) ESRD or DSCR, and 457 (17%) a CV event. Cox regression analysis showed that serum bicarbonate had a negative association with incident ESRD (HR 0.91 [95% CI 0.90-0.93], $P < 0.001$) and with the incidence of the combined endpoint of ESRD or DSCR (HR 0.94 [0.92-0.96], $P < 0.001$). These associations were independent of traditional risk factors for renal and CV disease, but lost significance when adjusting for baseline eGFR. Analysis of bicarbonate quartiles showed similar results for the lowest bicarbonate quartile (< 21 mEq/L) versus the quartile with normal bicarbonate levels (24-26 mEq/L). No associations were found between serum bicarbonate and CV outcome in any model. Of note, in these RCTs no interaction was found between treatment allocation and bicarbonate versus any of the three outcomes.

Conclusion: In this cohort of type 2 DM patients with diabetic nephropathy, serum bicarbonate was not independently associated with renal or CV endpoints. This finding suggests that the predictive value of serum bicarbonate for adverse outcomes differs between diabetic and non-diabetic patients.

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Association between 3 biomarkers and kidney complications in type 2 diabetic patients

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Background and aims: We aimed to explore the predictors of severe renal complications in type 2 diabetes in a multi-biomarker approach. Three peptides recently reported as associated with cardiovascular or renal complications of diabetes in the literature were considered: the mid-regional part of pro-ADM (MR-proADM) which is a surrogate marker of adrenomedullin, ultrasensible copeptin (usCT-proAVP) which is a surrogate marker for arginine vasopressin release and soluble Tumor Necrosis Factor receptor 1 (sTNFr1) which is a marker of the TNF pathway.

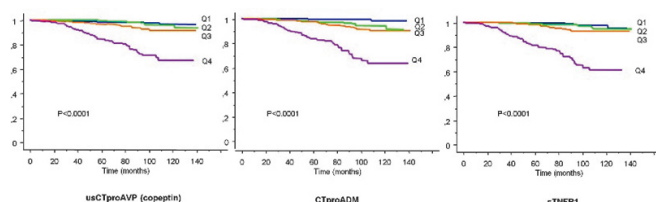
Materials and methods: A total of 1,408 T2D patients without history of renal replacement and GFR > 15 mL/min at baseline were prospectively followed in a mono-center hospital-based cohort in middle-western part of France. Median duration of follow up was 70 months (8,365 patient.years). The three peptides were measured on the same baseline plasma sample. Renal events were defined as sustained doubling of serum creatinine levels or renal replacement therapy during follow-up. Events were adjudicated by an independent adjudication committee.

Results: During follow up 82 patients yielded a renal event (9.9 for 1,000 patient.years). The median (interquartile range) MR-proADM, usCT-proAVP and TNFR1s plasma concentrations were 0.73 (0.36) nmol/L, 6.57 (7.68) pmol/L and 1843 (770) pg/L respectively.

In univariate analysis, MR-proADM, usCT-proAVP and TNFR1s were significantly associated with risk of renal event ($p < 0.0001$ for all). Hazard ratio (HR) [95%CI] were 1.17 [1.15-1.20], 1.02 [1.02-1.03] and 1.11 [1.09-1.12] for an increase of 0.1 nmol/L MR-proADM, 1 pmol/L usCT-proAVP and 100 pg/L TNFR1s respectively. Kaplan Meier survival curves according to quartiles groups of biomarkers are presented in Figure. MR-proADM, usCT-proAVP and TNFR1s were significantly inter-correlated (Spearman test, all $p < 0.0001$). When considering together all three biomarkers, only MR-proADM and TNFR1s remained significant predictors of renal event. After adjustment on age, sex and baseline eGFR, adjusted HR [95%CI] were 1.12 [1.07-1.17] and 1.05 [1.02-1.07] for an increase of 0.1 nmol/L and 100 pg/L of MR-proADM and TNFR1s, respectively.

Conclusion: High levels of MR-proADM and sTNFR1 were independently associated with an increased risk of renal event in patients with type 2 diabetes. With this approach, usCT-proAVP did not carry additional information regarding of renal event when adjusting on these 2 biomarkers.

Figure. Kaplan-Meier survival curves without renal event according to biomarkers quartiles (patient with eGFR<15ml/min and without ESRD n=1408)



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Lipoprotein(a) predicts new onset of chronic kidney disease in patients with type 2 diabetes mellitus: a ten-year follow-up study

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Background and aims: We investigated factors that might influence the development of chronic kidney disease (CKD) in patients with type 2 diabetes. **Materials and methods:** From January 2000 to December 2002, a total of 1,367 patients with type 2 diabetes without CKD (estimated glomerular filtration rate [eGFR] ≥ 60 ml/min/1.73m²) were consecutively enrolled. Patients were divided into two groups according to their baseline serum Lp(a) levels (Lp(a) >30mg/dL vs Lp(a) \leq 30mg/dL). The estimated GFR was measured annually, and new onset CKD was defined as eGFR < 60 ml/min/1.73m² using a Modification of the Diet in Renal Disease formula. We used a Cox proportional hazard regression analysis to test associations between new onset CKD and potential explanatory variables.

Results: Of the 1,367 patients who were enrolled in this study, 904 (66.1%) completed the follow-up evaluation. The median follow-up time was 9.8 years. The mean age was 56.0 ± 10.3 years, and the duration of diabetes was 8.5 ± 6.9 years. The baseline eGFR was 98.3 ± 25.6 ml/min/1.73m². During the follow-up period, 234 patients (25.9%) progressed to chronic kidney disease. The patients in the CKD group were older ($P < 0.001$), had hypertension ($P < 0.001$), a longer duration of diabetes ($P < 0.001$), higher baseline A1C levels (Lp(a) >30mg/dL vs Lp(a) \leq 30mg/dL: hazard ratio 3.4; 95% CI 2.50 - 4.54; $P < 0.001$) after adjusting for sex, age, diabetic duration, mean A1C, albuminuria, treatment of insulin, ACE inhibitor/ARB and lipid lowering agents.

Conclusion: In conclusion, the development of CKD from normal renal function was independently associated with serum Lp(a) level in patients with type 2 diabetes.

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Lipidomic biomarkers associated with and predictive of rapid progression of renal disease in type 2 diabetes

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Background and aims: To identify lipidomic biomarkers associated with and/or predictive of rapid progression of nephropathy in subjects with type-2 diabetes mellitus.

Materials and methods: 288 serum samples (134 cases and 154 controls) from the Genetics of Diabetes Audit and Research Tayside Study (Go-Darts) study were selected based on changes in eGFR following baseline sampling. All cases and controls had a baseline eGFR of 30-60ml/min with cases losing >40% of baseline eGFR over a maximum follow-up of 3.5 years, whilst controls lost a maximum of 5% baseline eGFR after >3.5 years follow-up. 221

lipid species, belonging to 9 lipid classes, were measured using a mass spectrometry platform. We assessed the association with the rapid eGFR loss with individual lipid species, the sum of the lipid class and ratios of individual lipids to the sum of lipid class, by univariate tests and using predictive selection models. We applied two complementary approaches to biomarker selection: forward selection using logistic regression, and sparse logistic regression with the L1 (LASSO) regularization penalty, with models adjusted for standard clinical covariates including age, sex, diabetes duration, baseline eGFR, albuminuria, HbA1c and use of ACE Inhibitors or ARBs. Model performance was assessed using Area Under the Receiver Operating Characteristic curve (AUROC) from cross-validated models.

Results: A model limited to clinical covariates only had an AUROC of 0.695. A class-by-class analysis of lipids reveals that the most predictive associations are with the Phosphatidylcholines lipids (PC) (AUROC = 0.739 with forward selection, 0.764 with LASSO-regularized selection). Other lipid classes which help improving predictions are Sphingomyelins (SPM) (AUROC = 0.724 and 0.726). Among the Phosphatidylcholines PC26.6 was inversely associated with progression in univariate analysis (Odds Ratio 0.66, 95% Confidence Interval 0.49, 0.86, $p < 0.001$).

Conclusion: Circulating serum phosphatidylcholines are associated with rapid eGFR loss in patients with diabetes and significantly improve its prediction. The mechanism underlying association warrants further investigation.

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Epidemiology of urinary tract infections in type 2 diabetes mellitus patients and associated healthcare cost

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Background and aims: Urinary tract infections (UTIs) are considered to be the most common bacterial infection in diabetes mellitus type 2 (T2DM) patients (pts). This study aims to assess the incidence of UTIs, identify predictors of UTI event risk, and describe the associated treatment costs in T2DM pts.

Materials and methods: This was a retrospective database analysis of the German sickness fund covering Year 2010-12. Patient inclusion criteria were documented continuous insurance from 2010-12 and documented ≥ 1 inpatient or ≥ 2 outpatient T2DM diagnoses. To be included as an UTI case, the UTI diagnoses had to be coded after a T2DM diagnosis. A UTI diagnosis was interpreted as incident if no prior UTI diagnosis was documented. If a prior UTI was documented, such a diagnosis was interpreted as recurrent only if it was not documented in the same or following quarter as the prior UTI AND documented by either a different physician and/or associated with antibiotics use. UTI event rates were calculated per 1,000-patient-years. Logistic regressions were used to evaluate the association between pts characteristics and the risk of UTI within the whole sample and within a subsample of pts with available HbA1C/BMI data. Costs included UTI-related antibiotics use, outpatient and inpatient treatment. Cost was analysed descriptively.

Results: There were 456,827 T2DM pts meeting the inclusion criteria. Median age was 75.5 years; 43.8% were male. In the first observation year (2010), 60.8% of the prevalent T2DM pts received antidiabetic therapy (OAD: 76.1%; insulin: 40.5%). Average Charlson comorbidity index (CCI) was 7.29. In the observation period 2010-12, 89,024 pts (19.5%) had ≥ 1 UTI, and 28,393 patients (6.2%) with ≥ 2 UTI. The event rate was 120 cases/1,000 patient-years. An inpatient admission occurred in 3.7% of the observed UTI events. In the multivariate analysis, the following variables, among others, predicted the risk to experience ≥ 1 event (OR; all with $p < 0.001$) in 2012: age 77-83 years and > 83 years vs. ≤ 62 years (1.46/1.58), female gender (1.66), previous UTI in 2010/11 (3.20), and other non-UTI infections in 2010/11 (1.74). Variables were mainly associated with the risk to experience ≥ 2 UTI in 2012: age 72-77, 77-83 years and > 83 years vs. ≤ 62 years (1.38/1.51/1.65), female gender (1.32), previous UTI in 2010/11 (5.13), other non-UTI infections in 2010/11 (1.63), and CCI > 8 (1.67). In the subgroup with laboratory data available (217,022 pts), a higher UTI risk in 2012 was additionally associated with a mean HbA1C of 9.5-10.0% vs. 7.0-7.5 in 2011 (1.31/1.33; $p < 0.001$). The most commonly prescribed antibiotic for outpatients was ciprofloxacin (48.8%). Mean (SD), and median costs per UTI case were 193.28 € (802.85 €) and 68.94 € respectively (inpatient: 50.5%; outpatient: 43.6%; medication/other: 5.9%).

Conclusion: Among a real-life T2DM cohort from 2010–12, UTI event risk was very high compared to previous studies which reported event rates 47–60 cases/1,000 patient years. This may be due to the older and more comorbid sample which reflects a real life cohort of T2DM patients in Germany. Age, female gender, health status and previous infections are the greatest risk factors associated with the UTI event risk.

OP 03 Evolving tools in education

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Internet intervention designed to identify and reduce risk of diabetic driving mishaps, a randomised clinical trial

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Background and aims: While driving mishaps are more common among those who have Type 1 Diabetes (T1D), in part due to extreme blood glucose (BG), this increased risk is believed to be restricted to a subgroup of drivers who are more vulnerable to moderate hypoglycaemia. We tested the hypotheses that an interactive internet intervention, DiabetesDriving.com (DD.com), could: 1) identify high-risk drivers, and 2) aid high-risk drivers to diminish their risk by better anticipating, preventing, detecting, and treating extreme BG while driving.

Materials and methods: 1739 drivers with T1D from all 50 US states who registered for DD.com were screened for inclusion criteria, and for High or Low risk for future driving mishaps based on the Risk Assessment for Diabetic Drivers (RADD). 379 High-Risk drivers were randomized to Routine Care (RC), DD.com, or DD.com + Motivational Interviews administered once before and once after DD.com. 122 Low Risk drivers were assigned to RC. DD.com was administered during months 1–2. During months 3–14, all participants completed monthly on-line driving diaries in which the frequency of the following driving mishaps was reported: Severe hypoglycaemia while driving, Loss of vehicle control without hitting anything, Collisions, Being stopped by the police while driving, Automatic driving due to extreme BG, Unintentional stops while driving, and Requiring assistance from someone else while driving due to extreme BG.

Results: Analyses of partial data (4,036 diaries) indicate High-Risk RC drivers reported more than twice as many driving mishaps/month than Low-Risk RC drivers (0.54 vs 0.21 mishaps/month, $p < 0.001$). Both DD.com groups reported similar numbers of mishaps/month (0.31 and 0.29), significantly fewer than for the High-Risk RC participants ($p < 0.001$). DD.com participants who answered “yes” to the question, “Do you use continuous glucose monitoring (CGM)?” reported a similar frequency of driving mishaps to those who said “no” ($p = 0.68$), but this may be because the CGM users had significantly greater driving risk (RADD scores) than the non-CGM users ($p = 0.026$).

Conclusion: Results indicate: 1) Diabetes-related driving mishaps are relatively high frequency events for high-risk routine care individuals, 2) RADD can identify T1D drivers at-risk for future mishaps, 3) DD.com can reduce, and possibly prevent, future driving mishaps of T1D drivers at high risk for mishaps, and 4) the additional costs of MI cannot be justified in this particular situation.

Clinical Trial Registration Number: NCT01563887

Supported by: NIDDKD R01

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Effects of peer support in type 2 diabetes patients on diabetes related distress, self efficacy and well being: a randomised controlled trial

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Background and aims: Many type 2 diabetes mellitus patients face difficulties self-managing their illness, which can lead to high levels of diabetes-related distress. Diabetes distress may be decreased by peer support, as peers understand and have dealt with similar problems, and can help motivate each other. However, a recent systematic review concluded that evidence of benefits of peer support in patients with type 2 diabetes mellitus were inconsistent. The primary aim of this study was to investigate the effectiveness of a group-based, peer support program on diabetes-related distress, well-being and diabetes-specific self-management in type 2 diabetes patients.

Materials and methods: A parallel group randomised controlled trial of a six session group-based peer support intervention, delivered by peer leaders and group psychotherapists, compared with one educational meeting on diabetes. Duration of the intervention was six months. Patients with a type 2 diabetes

duration of three years or more and between 50 and 70 years of age, recruited via their general practitioner, were randomised to receive the peer support intervention (n=101) or one educational meeting (n=132). Outcomes were measured one month before, and 6, and 12 months after baseline by means of self-reported questionnaires (PAID; WHO-5; diabetes specific CIDS). Baseline characteristics between intervention and control group were compared and multilevel linear regression models were performed on complete cases to investigate the effect of the intervention on diabetes-related distress (n=118), psychological well-being (n=116) and diabetes-specific self-management (n=107) (Mlwin2.22). Unadjusted analysis as well as analysis adjusted for differences in baseline characteristics are presented.

Results: Mean age of the study participants was 64 years (SD: 5) in both the control and intervention group. More men were randomised to the intervention group (69.3%) and more participants were lower educated (60.4%). Other characteristics did not differ between the two groups at baseline. No effects were seen for diabetes-related distress or well-being. However, the intervention group showed an increase in diabetes-specific self-management after 6 months of follow-up ($\beta=4.6$; 95% CI: -5.4 to 14.6) and between baseline and 12 months of follow-up ($\beta=5.1$; 95% CI: 1.2 to 9.0). Adjustment for differences in sex and educational level between intervention and control group at baseline slightly increased the differences in effects after 6 months ($\beta=6.5$; 95% CI: -2.8 to 15.7) and after 12 months ($\beta=7.0$; 95% CI: 3.01 to 11.02).

Conclusion: Results of this study indicate that a group-based, peer support program had no effect on diabetes distress nor on well-being but a modest beneficial effect on diabetes-specific self-management in type 2 diabetes patients was seen, which sustained for at least six months after the last peer group session.

Clinical Trial Registration Number: NTR3474

Supported by: Dutch Diabetes Research Foundation

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The efficacy of a new education and treatment programme (PRIMAS) for people with type 1 diabetes in daily routine and RCT: a health care research trial

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Background and aims: It's a common result of health care research that efficacy of treatments assessed in randomized trials (RCT) is superior to efficacy of these treatments in routine care. We evaluated if a new education and teaching programme for type 1 diabetes patients (PRIMAS) had a similar efficacy in daily routine than in the RCT.

Materials and methods: In this health care research trial (HCRT) 255 people with type 1 diabetes from 42 diabetologists practices participated (age 43 ± 14 yrs.; 54% male gender; diabetes duration 13 ± 12 years; HbA1c $8.1 \pm 1.4\%$). Participants took part in the education and treatment programme (PRIMAS), which consisted of 12 lessons. The outcomes were assessed 6 months after the education programme was finished. In both trials HbA1c was assessed in a central laboratory. Patients also completed a Hypoglycaemia Awareness Questionnaire, the Center of Epidemiological Studies-Depression Scale (CESD), the Diabetes Distress Scale (DDS), the Diabetes Self-efficacy Scale and the Empowerment Scale. Central outcomes were the difference in HbA1c reduction between the RCT and this health care research trial. Secondary outcomes were improvements of the hypoglycaemia awareness score, depressive symptoms, diabetes related distress, self-efficacy and empowerment.

Results: HbA1c showed an equivalent improvement in RCT and HCRT (-0.36 ± 1.1 vs. -0.39 ± 1.2 percentage points, $\Delta 0.03$ 95% CI -0.27 to 0.33 percentage points, $p=.827$). The confidence interval of HbA1c-differences was beneath the 0.4 % non-inferiority threshold as defined by the ADA. Similar results were obtained regarding the hypoglycaemia unawareness score (RCT -0.52 ± 1.42 vs. HCRT -0.36 ± 1.36 , $p=.375$), for depressive symptoms score (RCT -1.18 ± 7.93 vs. HCRT -1.85 ± 8.49 , $p=.542$), for diabetes distress score (RCT -0.32 ± 0.76 vs. HCRT -0.18 ± 0.66 , $p=.180$) for self-efficacy score (RCT $+1.39 \pm 3.56$ vs. HCRT -1.11 ± 5.88 , $p=.631$) and for empowerment score (RCT $+2.61 \pm 5.93$ vs. HCRT -2.34 ± 7.01 , $p=.752$).

Conclusion: The PRIMAS diabetes education and treatment programme had similar effects under routine care conditions than observed in a RCT. Thus, PRIMAS can contribute to an improvement of routine health care in people with type 1 diabetes.

Clinical Trial Registration Number: NCT01220557

Supported by: Berlin Chemie AG

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Effects of a patient-oriented decision aid for prioritising treatment goals in diabetes, a randomised controlled trial

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Background and aims: Decision aids (DA) can encourage patient-provider discussions about disease management by presenting available treatment options and expected outcomes for the individual patient. The newest generation DA for diabetes patients and providers prioritise between clinical domains to guide treatment decisions. Our aim was to assess the effects of such a patient-oriented DA in comparison to usual care on shared goal-setting and treatment decisions.

Materials and methods: We conducted a randomised controlled trial including 18 general practices in the north of the Netherlands. Four presentation formats of the DA were compared to usual care. Practices were randomly allocated to a computer-screen or printed version of the aid. Within each practice, patients were randomised to receiving either the short or extended version of the aid or usual care. Effects on patient empowerment for making shared decisions about treatment goals (primary outcome), smoking status, prescribing of glucose-regulating, blood pressure-regulating, lipid-regulating and albuminuria-regulating drugs (secondary outcomes) were tested in intention-to-treat and per-protocol analyses. Data were collected through structured questionnaires and automated data extraction from electronic health records in 6 months before and after the intervention.

Results: Of 665 eligible patients with type 2 diabetes being ≤ 65 years at time of diagnosis, 344 consented to participate; 225 were allocated to the intervention groups and 119 to the usual care group. The mean empowerment score increased 0.1 on a 5-point scale in the overall intervention group. This effect was not significantly different from the control group in the intention-to-treat analysis but was significant in the per-protocol analysis (n=103 patients, who received the DA as planned vs 119 control patients, $p=0.031$). Lipid-regulating drug treatment was intensified in 24% of intervention and 11% of control patients with elevated cholesterol levels, which was a significant difference in the intention-to-treat ($p=0.041$) as well as the per-protocol analysis ($p=0.038$). No significant changes were seen for the other drug treatments or in smoking status.

Conclusion: Our study showed that a patient-oriented treatment DA can lead to improved drug treatment and has potential for increasing patient empowerment, provided it is used as intended. The effects of the DA may have been limited due to its single use within the setting of this randomised trial.

Clinical Trial Registration Number: NTR1942

Supported by: ZonMW

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Health economic implications of education-based flexible insulin therapy versus conventional or technology-based approaches in type 1 diabetes

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Background and aims: To estimate the long-term clinical and cost outcomes associated with flexible intensive insulin therapy (FIT) in relation to standard management and a continuous subcutaneous insulin infusion with continuous glucose monitoring (CGM-CSII) for patients with type 1 diabetes in Switzerland.

Materials and methods: Evaluation of a long-term cost-effectiveness model with parameters estimated from three landmark type 1 diabetes trials: The Basel FIT study, STAR 3 and EDIC, with clinical outcomes extrapolated using the published and validated CORE Diabetes Model. Main outcome measures included life expectancy, quality-adjusted life expectancy, incidence of diabetes-related complications, direct costs, and incremental cost-effectiveness ratios (ICERs).

Results: FIT and CGM-CSII were associated with clinical benefits over standard management, improving life expectancy (14.43 years and 14.20 years versus 14.07 years) and quality-adjusted life expectancy (10.14 quality-adjusted life years [QALYs], and 9.97 QALYs versus 9.75 QALYs). Improvements were driven by reduced incidences of diabetes-related complications and severe hypoglycaemic events, as a result of improved glycaemic control

over standard management. FIT was associated with reduced direct medical costs over standard management (CHF 186,373 versus CHF 192,588), and therefore was considered to dominate standard management. Costs were higher in the CGM-CSII (CHF 245,983) arm as a result of increased costs of CSII pumps and CGM consumables, with an ICER of CHF 248,602 per QALY gained versus standard management.

Conclusion: The education-based approach of FIT and the technology-based approach of CGM-CSII are both likely to produce improvements in clinical outcomes, but the high cost of CGM-CSII represents a barrier to its use, particularly compared to the cost savings associated with FIT. Investment of physician time in patient education may be more cost-effective than investment in CGM-CSII technology, in terms of optimizing management of patients with type 1 diabetes in Switzerland.

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Effects of multidisciplinary and structured education programme on glycaemic control during transition of young adults with type 1 diabetes

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Background and aims: The transition from pediatric to adult diabetes care represents a high risk period for a person with diabetes. Prior studies highlight the risk of gaps in medical follow-up and adverse diabetes related outcomes in emerging adults, including poor glycaemic control, appearance of long term diabetes complications and early mortality. Very little is known about the specific aspects of transitions process, in particular about the role of structured education during transition. Aim of our study was to evaluate the relationship between multidisciplinary structured education during transition and post-transition glycaemic control.

Materials and methods: We evaluated baseline clinical characteristics, glycaemic control, glycaemic variability, hypoglycaemia frequency and their variation one year after multidisciplinary structured education program in 55 young adults with type 1 diabetes who have done transition process. Data for Glycaemic variability (SD of average glucose) and hypoglycaemia frequency were obtained from the analysis of glycaemic samples from personal blood glucose meters during a period of 90 days. Multidisciplinary structured education was done at the first visit (and carried on during the following months) by Diabetologist, Dietician and Nurse with particular attention to CHO counting, hypoglycaemia correction and modulation of insulin therapy during exercise. Every 2 months correct use of CHO count or adherence to constant CHO diet were verified by dietician. Descriptive statistics were presented as means and SD or proportions. Chi square test was used to compare proportions. Multivariate logistic regression model was used to verify the association between correct management of CHO and glycaemic control at 1 year (variable included: age, sex, Δ Hb1c Δ glycaemic variability, Δ hypoglycaemic frequency).

Results: Average population age was 27.8 ± 10.1 years with mean diabetes duration of 17.3 ± 9.9 years. Average transition GAP from the last pediatric diabetes visit and first adult visit was 6.24 ± 9.12 months. At the first visit to adult center mean Hb1c was $7.9 \pm 1.2\%$, on average worsen of $0.32 \pm 1.8\%$ during transition GAP. Glycaemic variability was 83.1 ± 23 mg/dl and hypoglycaemic frequency was $12.6 \pm 8.2\%$. Only 23.6% of patients correctly apply CHO counting and 11% employ constant CHO diet at baseline. Education was accepted by 85% of patients. One year later 34.6% of patients correctly apply CHO counting and 25.2% employ constant CHO diet ($p = 0.014$ and 0.037 vs baseline). Hb1c and glycaemic variability decreased significantly from baseline ($-0.72 \pm 0.65\%$ $p = 0.009$ and -12% $p = 0.041$ respectively). Hypoglycaemia frequency decreased not significantly (-2% $p = 0.124$). Correct CHO management was associated to reduction of Hb1c ($r = 0.293$ $p = 0.012$) less glycaemic variability ($r = -0.366$ $p = 0.021$) and lower hypoglycaemia frequency ($r = -0.534$ $p = 0.032$) after 12 months in univariate model but loose statistical relevance in multivariate one. Baseline Hb1c was the only predictor of glycaemic control after 12 months of follow-up ($R = 0.0659$ $p = 0.0001$).

Conclusion: In our study sample age of transition and transition GAP was high, during transition GAP glycaemic control worsen. A multidisciplinary structured education during transition, if accepted by patient, lead to a better CHO management that is associated to a better postransition glycaemic control.

OP 04 Brown adipose tissue

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Impaired brown adipose tissue fatty acid metabolism in obese subjects

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Background and aims: Brown adipose tissue (BAT) activation is commonly found during cold exposure: BAT glucose uptake and blood flow are increased in cold conditions. Obese subjects have a blunted glucose metabolism in BAT. The role of fatty acid metabolism in BAT function is uncertain. The aim of this study was to quantify NEFA uptake using PET-CT in lean and obese subjects during cold exposure and in room temperature (RT), and to measure the differences in NEFA uptakes between lean and obese subjects.

Materials and methods: NEFA uptake was quantified in lean ($n = 12$, 5F/7M, ages: 34.4 ± 11.8 years, BMI: 24.1 ± 1.4 kg/m²) and obese subjects ($n = 9$, 4F/5M, ages: 45.7 ± 5.8 years, BMI: 32.6 ± 4.4 kg/m²) using 18F-FTHA, a palmitate analog. Each subject was imaged twice, once at RT and once during cold exposure. Cold exposure was started 2 hours before the imaging session. Tissue specific NEFA uptakes were calculated for supraclavicular adipose deposits, deltoid muscles and subcutaneous white adipose tissue (WAT). A biopsy was performed to confirm the existence of BAT in the supraclavicular area. Subjects were grouped to BAT positive (BAT+) and BAT negative (BAT-) groups based on their biopsy result. Biopsy site was determined from CT images.

Results: Three lean subjects (2F/1M) had biopsy proven BAT. They had 7-fold higher NEFA uptake during cold compared to obese- subjects (5.56 ± 1.15 vs. 0.74 ± 0.26 $\mu\text{mol}/100\text{g}/\text{min}$, $P = 0.01$) and 4-fold compared to lean BAT- subjects (1.43 ± 1.00 $\mu\text{mol}/100\text{g}/\text{min}$, $P = 0.02$ vs. BAT+). Cold exposure increased BAT NEFA uptake in BAT+ subjects 3-fold compared to RT ($P = 0.02$). BAT+ subjects had higher BAT NEFA uptake in RT compared to obese subjects (1.69 ± 0.57 vs. 0.57 ± 0.55 $\mu\text{mol}/100\text{g}/\text{min}$, $P = 0.01$). BAT FA uptake in cold was higher in lean BAT- subjects compared to all obese subjects ($P = 0.01$). Lean and obese groups had similar muscle and WAT NEFA uptakes in cold and RT.

Conclusion: Cold stimulation increases NEFA uptake of BAT, but not skeletal muscles or WAT. All subjects who had functionally active BAT were lean, and all lean subjects had higher tissue activity during cold exposure in the supraclavicular area compared to the obese subjects. BAT+ subjects had increased BAT NEFA uptake in RT. This would suggest that BAT takes in NEFA even without cold exposure. These results suggest that obese subjects have an impaired NEFA uptake in the supraclavicular area, typical BAT region.

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Cold exposure increases the oxygen uptake rate of brown adipose tissue in humans

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Background and aims: Brown adipose tissue (BAT) activity has been suggested to have direct relation with insulin sensitivity and inverse association with obesity. BAT is more active during cold environment as it produces heat by the oxidation of glucose and fatty acids, a phenomenon known as non-shivering thermogenesis. Oxygen uptake rate can be a marker of BAT thermogenic potential as an indicator of oxidative metabolism. In our present study we aimed to determine whether cold exposure increases the oxygen uptake rate in BAT and how much BAT oxygen uptake rate is associated with blood flow and NEFA uptake rate during cold.

Materials and methods: Healthy lean and obese study subjects ($n = 8$, age: 34.5 ± 11.0 years, BMI range: $23.2 - 31.1$ kg/m²) of both genders (3F/5M) were studied at two different scanning sessions, 1) at room temperature (RT) and 2) with acute cold exposure, using PET-CT. Radioactive oxygen [¹⁵O]O₂, [¹⁵O]H₂O and [¹⁸F]FTHA were given for the measurements of oxygen uptake rate, blood flow and NEFA uptake rate of BAT, respectively. Indirect calorimetry was performed to assess the differences in whole body energy expendi-

ture between RT and cold environment. Data was compared with two tailed T-test and correlation analysis were performed with Pearson's correlations.

Results: Cold exposure increased oxygen uptake rate by 22.4 % (from 2.21 ± 0.68 to 2.85 ± 0.59 ml/100g/min, $P = 0.001$) in 16 BAT regions within 8 subjects, while oxygen uptake tended to be higher during RT in deltoid muscle (2.55 ± 1.52 vs. 3.13 ± 1.85 ml/100g/min, cold vs. RT, $P = 0.06$); however, no significant difference was found in white adipose tissue (1.76 ± 0.21 vs. 1.78 ± 0.19 ml/100g/min, cold vs. RT, $P = 0.76$). Cold exposure also tended to increase whole body energy expenditure (from: 7.07 ± 1.10 to 8.24 ± 2.43 MJ/24h, $P = 0.09$). During cold exposure, oxygen uptake rate correlated with blood flow ($r = 0.51$, $P = 0.05$) and NEFA uptake rate in BAT ($r = 0.76$, $P = 0.01$, $n = 5$).

Conclusion: High oxygen demand during cold in brown adipose tissue is most likely due to increased oxidation of available substrates (either glucose or fatty acids) for thermogenesis. Positive correlation between NEFA and oxygen uptakes suggests that NEFAs that enter brown adipocytes during cold also undergo β -oxidation within mitochondria.

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Loss of sympathetic drive may explain loss of brown adipose tissue activity in elderly but not in obese males

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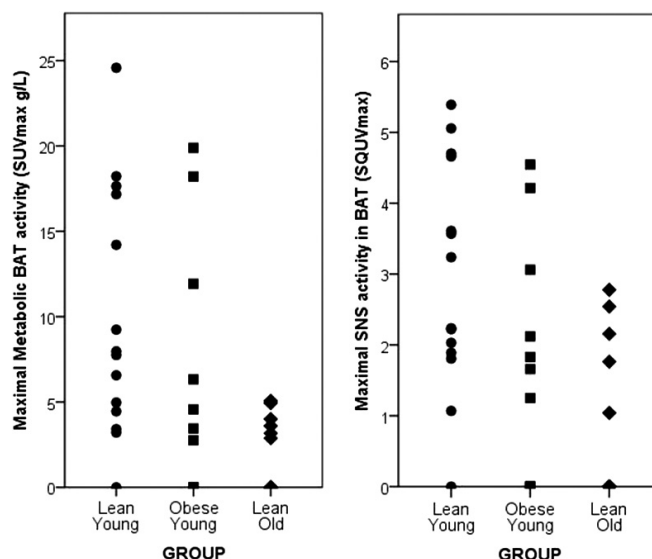
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Background and aims: Metabolically active brown adipose tissue (BAT) could facilitate weight loss by increasing energy expenditure. Cold is a potent stimulator of BAT, activating BAT primarily through the sympathetic nervous system (SNS). Old or obese individuals have less metabolic BAT activity than lean and young, but the role of the SNS in this decrease is unknown. We determined whether the lower metabolic BAT activity can be explained by a lower SNS response to cold.

Materials and methods: We studied 10 young obese (26 [21–31] years, BMI $32 [31–39]$ kg/m²), 10 old lean (55 [51–60] years, BMI $23 [22–25]$ kg/m²) and 14 young lean (26 [21–28] years, BMI $22 [21–23]$ kg/m²) males. Metabolic BAT activity was measured as maximal standardised uptake value and volume (SUVmax, SUVvol) on 18F-Fluorodeoxyglucose positron emission tomography CT (FDG). SNS activity was measured as semiquantitative uptake values of 123I-metaiodobenzylguanidine (MIBG) on single photon emission computed tomography scans, with the mediastinum as reference region. Scans were made after an overnight fast and 2 hours of cold exposure.

Results: Metabolic BAT activity and volume were different between young vs old (median [IQR] SUVmax (g/L) $7.9 [4.2–17.3]$ vs $3.0 [0.0–4.2]$ $p < 0.05$, SUVvol (cm³) $124.8 [10.9–333.8]$ vs $4.3 [0.0–12.3]$ $p < 0.05$) but unexpectedly, not for lean vs obese (SUVmax (g/L) $7.9 [4.2–17.3]$ vs $4.0 [0.0–13.5]$ $p = 0.2$, SUVvol (cm³) $124.8 [10.9–333.8]$ vs $11.8 [0.0–190.2]$ $p = 0.2$). HOMA-IR differed between lean and obese ($p < 0.01$) but not for young vs old. There was no correlation between HOMA-IR and either SUVmax or SUVvol. There were no differences in the SNS activity in BAT between lean and obese males ($p = 0.2$) but for young and old males this was borderline significant ($p = 0.06$). We confirmed the strong positive correlation between BAT activity measured with FDG and MIBG in the whole group (spearman correlation $\rho = 0.76$, $p < 0.01$), which we previously showed.

Conclusion: We conclude that loss of sympathetic drive may explain the loss of BAT activity in elderly but not in obese males.



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Seasonal variation in temperature influences acute brown adipose tissue activation

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Background and aims: Brown adipose tissue (BAT) has gained a lot of interest because of its capacity to convert calories into heat. Retrospective data on diagnostic 18F-Fluorodeoxyglucose (FDG)-Positron-Emission-Tomography (PET)-CT scans, in warm temperatures, show increased BAT activity during the winter months as compared to the summer months. Indeed, cold has been shown to be the strongest activator of BAT activity so far. Besides seasonal changes in BAT activity on diagnostic 18F-FDG-PET-CT scans, there is also an acute BAT activation after direct cold exposure. The magnitude of this acute effect might still be influenced by seasonal temperatures through preconditioning of BAT. Indeed, frequent exposure to cold has been shown to increase BAT activity and volume. The aim of this study was to evaluate the seasonal effect of BAT in subjects exposed to acute cold.

Materials and methods: 18F-FDG-PET-CT scans of 63 consecutive subjects were included. These subjects participated in BAT research in the Academic Medical Center between January 2010 and December 2013. All subjects were cold exposed at 16–17 °C in an air cooled room, after an overnight fast. Temperature data (24 hours, 1 week, 2 weeks and 4 weeks before the scan) were collected from the national weather institute. Correlations between BAT activity (i.e. maximal and mean standardised uptake value (SUVmax and SUVmean) and volume) and outdoor temperature (OT) were calculated by the Spearman's correlation coefficient.

Results: Forty-nine subjects were BAT positive. The BAT positive subjects were significantly younger (median age 25 IQR [21.1–29.4] vs 29.5 [23.5–51.8] years, $p < 0.05$) and leaner (median BMI $22.6 [21.3–23.7]$ vs $26.2 [23.3–32.6]$ kg/m², $p < 0.05$) as compared to the BAT negative subjects. One week and 4 weeks prior to the scan the OT was significantly lower in the BAT positive group compared to the BAT negative group: 10.0 °C ([4.6–16.4] vs 17.1 [9.1–17.6] °C, $p < 0.01$) and 10.6 °C ([7.7–16.3] vs 16.5 [10.3–17.0] °C, $p < 0.05$). However, 24 hours and 2 weeks prior to the scan there were no significant differences in OT between the BAT positive and BAT negative subjects. There was a strong negative correlation for both the maximal BAT activity as well as BAT volume and outdoor temperature for all different time points (24 hours, 1 week, 2 weeks and 4 weeks before the scan all $p < 0.01$).

Conclusion: These data confirm the strong negative correlation between outside temperature and BAT activity and volume. In addition the data show that BAT activation by acute cold is largely influenced by outside temperatures. It seems therefore advisable to study BAT activity during periods of relative cold.

Correlation Coefficients	SUVmax	SUVmean	BAT Volume
Temp 24 hours	-0.45 ($p < 0.001$)	-0.46 ($p < 0.001$)	-0.43 ($p < 0.001$)
Temp 1 week	-0.50 ($p < 0.001$)	-0.52 ($p < 0.001$)	-0.45 ($p < 0.001$)
Temp 2 weeks	-0.45 ($p < 0.001$)	-0.47 ($p < 0.001$)	-0.41 ($p < 0.001$)
Temp 4 weeks	-0.46 ($p < 0.001$)	-0.49 ($p < 0.001$)	-0.44 ($p < 0.001$)

Table 1. Spearman correlation coefficients between maximal BAT activity (SUVmax), mean BAT activity (SUVmean) BAT volume and Temperature data in the hours or week(s) before the scan.

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Brown adipose tissue activity associates with insulin sensitivity and liver adiposity independently of visceral fat mass in healthy adults

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Background and aims: Brown adipose tissue (BAT) activity associates with insulin sensitivity and inversely with obesity. However, it remains unclear if the association between BAT and insulin sensitivity is mediated by low intra-abdominal adipose tissue mass and low liver adiposity in subjects with active BAT. In this study, we examined associations between BAT activity during cold exposure and glycaemic control, whole body insulin sensitivity, intra-abdominal adipose tissue mass and liver adiposity.

Materials and methods: 22 healthy adult subjects (M/F: 8/14, 41.0 ± 8.3 years, BMI 27.3 ± 5.3 kg/m²) underwent positron emission tomography with [¹⁵O]-H₂O to measure BAT perfusion and [¹⁸F]-FTHA to measure NEFA uptake in the supraclavicular BAT depot during cold exposure. Cold exposure had started two hours prior to and continued during the PET imaging. Whole body insulin sensitivity (hyperinsulinemic euglycemic clamp) and HbA_{1c} were measured. MRI imaging was used for the measurement of intra-abdominal adipose tissue volume and liver adiposity was adjusted with MR spectroscopy.

Results: Cold-induced NEFA uptake in BAT was 2.25±1.75 μmol/100g/min and perfusion was 22.0±27.0 ml/100g/min. HbA_{1c} was 5.4±0.4%, M value was 6.8±3.2 mg/kg/min, visceral adipose mass was 3.3±2.0 kg and liver fat content was 5.7±6.9%. NEFA uptake in BAT correlated with whole body insulin sensitivity ($r = 0.50$, $P = 0.02$), intra-abdominal fat mass ($r = -0.43$, $P = 0.04$) and liver adiposity ($r = -0.47$, $P = 0.04$). Cold-induced BAT perfusion correlated with HbA_{1c} ($r = -0.57$, $P = 0.01$). These associations (perfusion vs. HbA_{1c} and NEFA uptake vs. whole body insulin sensitivity) were independent of intra-abdominal fat volume or liver adiposity (P always < 0.05). Associations between NEFA uptake and liver adiposity were also independent of intra-abdominal adipose tissue volume ($P = 0.03$).

Conclusion: These results show that cold-induced BAT activity is associated with glycaemic control and insulin sensitivity independently of visceral adipose tissue mass and liver adiposity in health. Subjects with high BAT activity might have lower visceral fat mass due to increased energy expenditure. Additionally, BAT NEFA uptake might prevent fat accumulation in liver in visceral obesity. Although, liver fat content and visceral obesity correlate inversely with BAT activity, BAT may associate with insulin sensitivity via a separate mechanism. These may include yet unrecognized BAT-derived cytokines affecting muscle metabolism.

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Inorganic nitrate promotes the browning of white adipose tissue through the nitrate-nitrite-nitric oxide pathway

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Background and aims: Inorganic nitrate is considered an oxidation end-product of nitric oxide with little biological activity. However, recent studies demonstrate that dietary nitrate, largely derived from green leafy vegetables, modulates mitochondrial function in man and is effective in reversing features of the metabolic syndrome in mice. The role of nitrate in white adipose tissue (WAT) function remains poorly characterized. The development of a brown-like phenotype in white adipocytes ("beige" or "brite" cells), a process known as "browning", includes the induction of thermogenesis, the dissipation of chemical energy as heat. Activation of the browning response in WAT may represent a process underlying the altered systemic energy balance observed with nitrate treatment.

Materials and methods: Wistar rats were treated with 0.35 mM, 0.7 mM or 1.4 mM sodium nitrate (NaNO₃) in drinking water for 18 days ($n = 6$ /group). Nitrate induced browning of WAT was assessed using both RT qPCR and mass spectrometry based metabolomics. In vitro browning of primary

adipocytes treated with 25 μM, 50 μM and 500 μM NaNO₃ was analysed using respirometry, metabolomics, stable-isotope labelling techniques and RT qPCR. The mechanism of nitrate induced browning was defined in primary white adipocytes using pharmacological inhibitors of nitric oxide (NO), guanylate cyclase and protein kinase G (PKG). The effect of hypoxia on nitrate induced browning of WAT was determined in vivo in Wistar rats and in vitro using primary adipocytes.

Results: Nitrate dose-dependently increased the expression of brown-adipocyte specific genes in WAT of nitrate treated rats (UCP-1, CIDEA, PGC-1α, CYCS, CPT1, ACADvl, Two-way ANOVA, Control vs. 0.35 mM NaNO₃ $P \leq 0.01$, Control vs. 0.7 mM NaNO₃ $P \leq 0.0001$, Control vs. 1.4 mM NaNO₃ $P \leq 0.001$). Metabolomic analysis demonstrated that treatment with nitrate also decreased the total triacylglycerol content within WAT (0.95-fold, 0.35 mM NaNO₃; 0.94-fold, 0.7 mM NaNO₃; 0.98-fold, 1.4 mM NaNO₃; ANOVA, Control vs. 0.35 mM $P \leq 0.01$, Control vs. 0.7 mM $P \leq 0.01$). These findings were reproduced in vitro using primary white adipocytes treated with nitrate and analysed using RT qPCR, stable isotope labelling and metabolomics, and were accompanied by an increase in oxygen consumption, assessed by respirometry (Control 4.2 nmoles O₂/min/106 cells, 50 μM NaNO₃ 6.6 nmoles O₂/min/106 cells, 500 μM NaNO₃ 7.7 nmoles O₂/min/106 cells, ANOVA, $P = 0.02$). Using pharmacological inhibitor assays in primary white adipocytes, nitrate was found to induce these phenotypic changes in WAT independently of NO synthase, through the recently identified xanthine oxidoreductase catalysed nitrate-nitrite-NO pathway and downstream cGMP/PKG signalling. Furthermore, the nitrate induced browning of WAT, both in vitro and in vivo, was enhanced in hypoxia.

Conclusion: Since beige/brite cells exhibit anti-diabetic and anti-obesity effects and WAT from obese individuals is characterized by hypoxia, nitrate may be an effective means of targeting the induction of browning to white adipocytes located in obese and hypoxic adipose depots to treat the metabolic syndrome.

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OP 05 Factors driving islet cell development

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G1 lengthening promotes pancreatic progenitor cell differentiation in mouse embryonic development

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Background and aims: Cellular-based therapies for diabetes mellitus, such as the differentiation of human embryonic stem cells (hESCs), require an in-depth understanding of pancreatic development. During early pancreatic development, the Pdx1+Cpa1+ tip multipotent progenitor cells give rise to all three cell types of the pancreas, exocrine, endocrine and ductal cells, while the trunk Pdx1+Cpa1- cells give rise to endocrine or ductal cells only. The process that regulates the proliferation and differentiation of these progenitor populations is not fully known. There is evidence during neurogenesis that the length of the G1 phase of the cell cycle can directly influence the differentiation of neural precursors. Thus, we hypothesize that the cell cycle length regulates pancreas organogenesis.

Materials and methods: Pregnant CD-1 mice (P12.5) were injected with the thymidine analog 5'Ethynyl-2'-deoxyuridine (EdU) every 1.5 hours starting at 9 am. Embryos were collected at evenly spaced intervals from 9:30 am to 8 pm. The number of Pdx1+Cpa1+ and Pdx1+Cpa1- cells labelled with EdU was determined using immunofluorescence and confocal microscopy (n>4). The lengths of the G1, S and G2/M phases were determined mathematically from the length of time required to label all dividing cells, the number of cells labeled at time 0, and the proportion of dividing cells.

Results: We determined that the G1 length of multipotent progenitor cells at E12.5 was 3.6 hours while the bi-potent trunk cells had a G1 length of 5.8 hours. We did not find any significant change in the lengths of either the S or G2/M phases of the cell cycle. Consistent with findings in neural development, the difference in total cell cycle length of these two progenitor populations at E12.5 was largely due to a lengthening of the G1 phase. Intriguingly, the maximal proportion of EdU labeled cells or the growth fraction was significantly different (p<0.05; n=17) between multipotent and bi-potent progenitor cells with 90% and 66% EdU+, respectively. Preliminary data suggests that the majority of non-dividing Pdx1+ cells are still located within the pancreatic epithelium, express other progenitor cell markers such as Sox9 and Nkx6.1, and are Ki67+. These results suggest that there is a population of progenitor cells that are still in the cell cycle but are not dividing. As it has been previously shown that Ngn3 expression in endocrine progenitors inhibits proliferation by activation of Cdkn1a we also examined whether Ngn3 cells are labelled with EdU. Consistent with these results, we did not find a meaningful population of Ngn3+EdU+ cells.

Conclusion: These results support the 'cell cycle length hypothesis' from neurogenesis and suggest that G1 lengthening is important for pancreatic progenitor cell differentiation. Understanding this process may give novel insight into pancreatic development and inform studies aiming to produce mature beta cells from hESCs for diabetes treatment.

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Up-regulation of MafA drives embryonic progenitor-derived insulin-producing cells towards maturation and functional improvement both in vitro and in vivo

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Background and aims: Shortage in pancreas donors has impeded transplantation therapy for insulin-dependent diabetes. Our previous work has generated insulin-producing cells from mouse embryonic stem cells and embryos-derived progenitor cells, which could secrete insulin upon glucose stimulation and correct hyperglycaemia after transplantation into diabetic animals. However, these cells failed to function completely alike the adult β -cells and revealed features of immaturity. It is known that MafA (v-maf musculoaponeurotic fibrosarcoma oncogene homolog A) acts as a master transcription factor in regulating β -cell development and glucose responsive-

ness, but its expression is significantly lower in our insulin-producing cells. This study aimed to investigate if up-regulation of MafA could improve the function of these cells.

Materials and methods: MafA-expressing lentivirus was used to transduce mouse embryonic progenitor-derived insulin-producing (MEPI)-1 cells, followed by evaluations of cell functions both in vitro and in vivo.

Results: MafA levels in MEPI-1 cells could be significantly elevated near to that in isolated adult islets by the lentivirus. Insulin production was augmented by 50% and the expression of many genes (e.g. insulin, GLP-1 receptor, Glut2, glucokinase, Kir6.2 and Nkx6.1) important for β -cell development and function was significantly enhanced. These cells also exhibited higher extents of glucose metabolism, membrane potential depolarization and intracellular Ca^{2+} concentration rise, indicating more active signaling events upon glucose stimulation. Particularly, MafA up-regulation markedly improved glucose-stimulated insulin secretion by reducing basal insulin release and shifting the dose-response curve of insulin secretion upon glucose stimulation more close to the physiological pattern. When transplanted into streptozotocin-induced diabetic mice, MafA-upregulated MEPI-1 cells were able to correct hyperglycaemia for a longer term and reduce the hypoglycaemic incidences. In addition, MafA up-regulation slowed down MEPI-1 cell growth both in vitro and in vivo, and arrested the cells at G1 phase, which might be due to the observed elevation of expression of p27, an important cell cycle inhibitor.

Conclusion: Up-regulation of MafA levels in MEPI-1 cells enhances expression of the β -cell relevant gene profile, increases insulin biosynthesis, improves stimulated insulin secretion by augmenting signaling cascade, slows cell growth, ameliorates their in vivo capability of correcting hyperglycaemia in diabetic animals. These findings indicate that MafA can promote maturation of embryonic stem cell-derived insulin-producing cells, enabling them to achieve better correction of hyperglycaemia for treatment of type 1 diabetes.

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Elucidating the role of Menin during islet cell development in the human foetal pancreas

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Background and aims: The transcription factor, Menin, a tumour suppressor encoded by the Men1 gene, has recently been implicated as necessary for murine pancreatic development, suggesting a dual functionality: (1) to promote endocrine (including progenitor) differentiation via Ngn3 expression during development; and (2) to suppress proliferation and insulin hypersecretion in the adult pancreas. Most studies of Menin in the human have focused on its neoplastic role in the adult pancreas; however, knowledge of Menin's function during human fetal pancreatic development is limited. In the present study, the expression pattern and functional role of Menin was examined in the early to mid-gestation human fetal endocrine pancreas.

Materials and methods: The presence of Menin in the human fetal (8-21 weeks fetal age) pancreas was characterized by quantitative RT-PCR, western blotting and immunohistological approaches. Isolated human fetal islet-epithelial cell clusters (15-18 weeks) were treated with either MEN1 siRNA or MEN1 overexpression vector.

Results: Immunostaining revealed strong nuclear Menin (nMenin) expression within pancreatic cells from 8 to 21 weeks of fetal age that paralleled Menin mRNA and protein expression patterns. nMenin+ cells co-localized with transcription factors (PDX-1, SOX9, NGN3) that are critical for maintenance of the progenitor pool and endocrine differentiation. A high proportion of Ki67+ cells contained nMenin signals at 8-16 weeks but this decreased significantly at 18-21 weeks of development; the proliferative capacity of nMenin+ cells was reduced in parallel. Knockdown of MEN1 in human fetal islet-epithelial cells significantly increased apoptosis, reduced proliferation and decreased SOX9, NKX2.2 and NKX6.1 expression levels. Overexpression of MEN1 had opposite results: a significant increase in cell proliferation and insulin mRNA, suggesting that Menin plays a positive role in the developing human fetal pancreas.

Conclusion: We have investigated the expression and potential function of MEN1 in the early to mid-gestation human fetal pancreas. Functional knock-down and overexpression studies both suggest that Menin promotes human fetal pancreatic cell survival and proliferation, maintains the islet progenitor pool and regulates islet differentiation, very different from its anti-tumorigenic role in the adult pancreas.

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SOX9 and WNT signalling during human foetal pancreatic development

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Background and aims: Regulation of pancreatic progenitor proliferation and differentiation is crucial when generating an appropriate beta-cell mass. SOX9 is an important factor in pancreatic progenitor maintenance as well as endocrine differentiation; however, the signaling pathways that regulate expression of SOX9 remain unclear. A study of murine duodenal epithelium demonstrated that Wnt signaling regulated progenitor expansion and differentiation in addition to Sox9 expression. In the present study, the co-localization and inter-relationship between SOX9 and Wnt/ β -catenin factors and their targets in the developing human pancreas were examined.

Materials and methods: Human fetal pancreata (8–21 weeks fetal age) were examined for SOX9 and Wnt signaling molecules using immunofluorescence, western blot and qRT-PCR approaches. Isolated human fetal (18–21 week) islet-epithelial cell clusters were also treated with or without recombinant WNT3A in a dose- and time-dependent fashion. In addition, cells were treated with either a GSK3 β inhibitor (1-Akp) or a Wnt signaling inhibitor (FZD8-CRD).

Results: Half of the SOX9+ cells expressed WNT3A at 8–12 weeks but the numbers decreased with age ($p < 0.05$ – 0.01); in contrast, FZD and β -catenin expression in SOX9+ cells remained stable. The majority of insulin+ cells expressed WNT3A, FZD and β -catenin throughout this developmental period. To examine WNT signalling, isolated human fetal (18–21 week) islet-epithelial cell clusters were treated with rWNT3A or vehicle. Cells treated with WNT3A showed a dose-related increase in β -catenin localization. In addition, a significant increase in SOX9 and ISL1 expression as well as the number of insulin+ cells was observed in the WNT3A treated group after 48h of culture ($p < 0.01$ vs. controls), but no significant effect on the glucagon+ cell number. Cell proliferation studies using Ki67 labeling showed a transient increase in cell proliferation at 4h of culture but no changes in proliferative capacity at 24 and 48h. Similar results were observed when the cells were treated with a GSK3 β inhibitor. Furthermore, these effects of WNT3A on human islet-epithelial cell differentiation could be partially blocked by co-treatment with FZD8-CRD, a Wnt signaling inhibitor, suggesting that WNT3A induces SOX9 expression and beta cell differentiation through both FZD and non-FZD pathways.

Conclusion: These data indicate an active role for WNT signalling during endocrine cell differentiation in the early to mid-gestation human fetal pancreas. SOX9+ progenitors express WNT3A, FZD and β -catenin, suggesting that the WNT pathway may be involved in maintenance and differentiation of these cells. The present data provide evidence for a role of Wnt/ β -catenin and SOX9 in determination of insulin cells by regulating proliferation versus differentiation.

Supported by: CDA

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Changes in microRNAs profile underline the dedifferentiation process of in vitro cultured human pancreatic islet cells

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Background and aims: Beta-cell dedifferentiation has recently been identified as a mechanism of beta-cell dysfunction both in type1 and type2 diabetes. Upon metabolic or inflammatory stress, beta-cells undergo a specific phenotypic re-arrangement which on one side protects them from apoptosis and, on the other, elicits functional impairment. Previously, we have set up

a model of in vitro de-differentiation of human native islets (HI) that, when cultured in appropriate conditions, undergo an epithelial-mesenchymal like transition process (EMT), generating human pancreatic islet-derived mesenchymal cells (hPIDM). MicroRNAs (miRNAs) are smallRNAs, which regulate gene expression. MiRNAs have been demonstrated to control several biological processes including stemness and cell differentiation. MiRNAs have also been proposed to contribute to the development of many disorders including diabetes. Here, we aimed at characterizing the miRNA expression profile during human pancreatic islet cell dedifferentiation process.

Materials and methods: Human native islets were collected from 3 multiorgan donors and cultured for 15 days. De-differentiated proliferating hPIDM cells were collected and total RNA was extracted from human islets and from hPIDM cells. Expression of 768 miRNAs was evaluated using Taqman Human miRNAs panel A and B arrays. MiRNA prediction target analysis followed by gene ontology classification was performed using Targetscan and DAVID 6.7. MiRNA target genes expression levels on 3 different human native islets preparations and 3 hPIDM cells samples were evaluated with Taqman plates by using Real Time PCR. Data and statistical analysis was performed using expression suite software

Results: Among 768 miRNAs analyzed, 335 and 331 miRNAs were detected in HI and in hPIDM cells respectively. Following dedifferentiation, 110 miRNAs resulted significantly decreased and 13 increased in hPIDM cells vs native human islets. Upregulated miRNAs included miR-100, miR-337-3p, miR-214, miR-199a-3p and -5p, miR-137, miR-708, miR-99a and miR-302s. To gain insights into the molecular pathways potentially regulated by these miRNAs, we looked at the predicted targets genes. Using Targetscan, we extrapolated a list of 196 genes predicted as targets of the 13 upregulated miRNAs. In order to classify the predicted genes we used the algorithm DAVID 6.7 which allowed us to perform a gene ontology analysis. We identified 11 functional categories with a significant p-value. Among these categories, most genes belonged to cell-cell adhesion mechanisms and to differentiation process; By analyzing the expression levels of a selection of these predicted target genes in HI and hPIDM, we observed a major downregulation of 44 genes during EMT, in line with the inverse expression levels of targeting miRNAs

Conclusion: We detected a specific miRNA signature of in-vitro dedifferentiated human pancreatic islet-derived mesenchymal cells. We specifically identified 13 miRNAs strongly upregulated during this process, which may indeed function as regulators of those genes controlling mesenchymal-like phenotype acquisition. Using a bioinformatic approach we uncovered a putative specific role for these miRNAs in exploiting several functions by regulating the expression of genes involved in cell-adhesion, cell-cell contact, morphogenesis and in mesenchymal-like phenotype acquisition.

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Transcriptome profiling by RNAseq of single human islet cells reveals unique features of individual alpha and beta cells

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Background and aims: Deep coverage RNA sequencing (RNAseq) allows for the detailed description of transcriptomes. Typically this is performed on pure or mixed cell populations and the data offer no insight into the transcriptome signature or true identity of individual cells, or cell-to-cell variability in gene expression. To understand the unique features of individual human islet cells, we have now captured single cells and analysed them by RNAseq.

Materials and methods: Human islets isolated from 2 healthy donors (samples C1, C2) and 2 with type 2 diabetes (D1, D2) were dissociated (accutase) and single cells captured and processed in 96-well microfluidic plates (Fluidigm C1 Single-Cell Auto Prep System). We RNA sequenced 288 single cells with Illumina HiSeq2000 (100 base pairs, paired-end reads), with an average of 36.6 million total reads. mRNA levels are expressed as Reads Per Kilobase per Million mapped (RPKM).

Results: After quality control, 275 cells were analysed. Of these, 123 were considered beta cells (defined as RPKM INS (insulin) > 100 , GCG (glucagon), PPY (pancreatic polypeptide) and SST (somatostatin) < 100). The number of beta/total cells for each donor was: C1: 42/68; C2: 50/81; D1: 14/63; D2: 17/63. In these cells, INS was highly expressed, but with considerable inter-cellular variability (RPKM range for C1: 1040–52410; C2: 167–104500; D1: 293–35930; D2: 316–22440). Interestingly, there was expression of GCG in all beta cells (range 1–75 RPKM), albeit at much lower levels than in the 48

captured alpha cells (RPKM GCG >100, INS, PPY and SST <100) with RPKM range C1: 68530–175400, C2: 36670–188100; D1: 110–90340; D2: 5752–59780. Similarly, there was low INS expression in all alpha cells (range 6–89 RPKM). Analysis of empty wells indicated that this low-grade expression was not due to contamination. There is considerable interest in islet cell plasticity, particularly possible alpha-to-beta cell trans-differentiation. We identified a total of 53 mixed phenotype “alpha-beta” cells (RPKM INS and GCG >100, PPY and SST <100) distributed as: C1: 6; C2: 10; D1: 25; D2: 12 cells, with INS 107–70790 and GCG 115–136500 RPKM. The ratio INS/GCG also varied: 0.004–119 (C1), 0.12–471 (C2), 0.009–222 (D1), 0.003–2.20 (D2). It has not yet been possible to determine whether the GLP-1 receptor (GLP1R) is expressed in human alpha as well as beta cells, yet this is of great clinical interest. Just 19 of the 123 beta cells expressed detectable levels of GLP1R (C1: 6/42 cells, 0.03–79.4 RPKM; C2: 7/50, 0.03–14.5; D1: 3/14, 0.04–70.9; D2: 3/17, 0.03–0.49), whereas no alpha cells expressed any detectable GLP1R.

Conclusion: We have for the first time analysed gene expression in single human islet cells. There is remarkable intercellular variability in INS and GCG expression that may reflect beta and alpha cell functional heterogeneity. Intriguingly, the regulation of expression of these two genes is not absolute, with low expression of INS in alpha and GCG in beta cells. There were several “alpha-beta” cells in all preparations, possibly representing cells in transition from one type to another. GLP1R was expressed in some but not all human beta cells, but was not detectable in any of the alpha cells. Inability to detect GLP1R expression could in theory be due to inadequate sequencing depth but in any event indicates extremely low levels of mRNA in all alpha and many beta cells that may not translate to physiologically meaningful protein levels.

OP 06 Novel mechanism of glucose tolerance

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Withdrawn

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Brain natriuretic peptide prevents diet-induced obesity and glucose intolerance in mice

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Background and aims: Growing evidences indicate that natriuretic peptides (NPs) and downstream cyclic GMP-dependent signaling play an important role in the regulation of energy metabolism. Recent studies have highlighted relationships between reduced circulating NPs levels, obesity and the risk of type 2 diabetes (T2D). The present work aimed to demonstrate the pathophysiological link between NPs deficiency and metabolic disturbances in different mouse models of obesity and T2D.

Materials and methods: We first measured natriuretic peptides receptor (NPRA and NPPC) protein expression by western blot in genetic obese/diabetic mice (db/db versus db/+). We next investigated the effect of continuous delivery of Brain-NP (BNP) via intraperitoneal osmotic mini-pumps for 4 weeks in db/db and high fat diet (60% kcal)-fed mice. Metabolic parameters such as body weight, body composition, insulin and glucose tolerance were examined.

Results: NPRA/NPPC protein ratio (NPs biologically active receptor/clearance receptor) was dramatically down-regulated in skeletal muscles, liver and white and brown adipose tissue in db/db mice compared to db/+ mice ($p=0.0001$). More interesting, chronic BNP infusion improved glycaemic control reflected by reduced HbA1c (-15% , $p=0.01$) in db/db mice, and prevented body weight gain (-45% , $p=0.004$) and improved glucose tolerance ($p=0.02$) in HFD-fed mice compared to saline-treated mice. This better metabolic profile was accompanied by increased palmitate oxidation in skeletal muscle ($p=0.04$) and reduced lipotoxicity reflected by reduced intramyocellular content of C18-diacylglycerols (-15% , $p=0.08$) and total ceramides (-17% , $p<0.05$).

Conclusion: Collectively, our data indicate that obesity is associated with a reduced biological activity of NPs in several metabolic organs such as skeletal muscles that may contribute to the development of T2D. Increasing plasma NPs levels improves glycaemic control and glucose tolerance in obese/diabetic mice. Some of these beneficial effects may be driven by improved lipid oxidative capacity and reduced lipotoxic lipid pressure in skeletal muscle. Supported by: ANR-12-JSV1-0010-01 and Société Francophone du Diabète

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Insulin is dispensable for the glucose lowering action of leptin in mice with streptozotocin-induced diabetes and congenital insulin deficiency

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Background and aims: It is widely believed that insulin is the only hormone capable of normalizing the catabolic consequences of type 1 diabetes. However, it has been shown that the hormone leptin can reverse hyperglycaemia in streptozotocin (STZ)-induced diabetes in the absence of insulin therapy. All previous reports of leptin treatment in insulin deficient rodents used chemical or immune mediated β -cell destruction, where insulin depletion is incomplete. Since leptin enhances insulin sensitivity, we sought to definitively determine whether leptin can reverse hyperglycaemia independently of insulin.

Materials and methods: To determine the effect of insulin receptor antagonism on leptin action, STZ-treated mice were implanted with a pump delivering either vehicle or 20 $\mu\text{g/day}$ leptin or an insulin pellet to control fasting blood glucose. Fasted mice were administered an i.p. injection of vehicle or 25 nmol/kg of the insulin receptor antagonist S961 alone (day 5) or preceding an oral glucose gavage (day 7), and subsequent glucose homeostasis was assessed. In addition, we tested whether leptin treatment in mice with con-

genital insulin deficiency could extend lifespan and reverse hyperglycaemia. *Ins1^{-/-}Ins2^{-/-}* (InsKO) mice were maintained on twice daily insulin injections until 14 days of age then transplanted with ~100–150 islets into the anterior chamber of the eye. At ~10 weeks of age InsKO mice were treated daily with an i.p. injection of 10 µg/day PEG-ylated leptin or vehicle and vehicle treated *Ins1^{-/-}Ins2^{+/-}* (Het) littermates were used as controls. On day 4 of treatment the transplanted eye was enucleated to render the mice entirely insulin deficient. Survival, body weight, and fed and fasting blood glucose were assessed.

Results: In STZ-treated mice both leptin and insulin treatment reduced fasting blood glucose by day 2 compared to vehicle treated controls (22.6±0.7 mM STZ-vehicle, 10.8±2.0 mM STZ-leptin, 12.5±2.0 mM STZ-insulin). Injection of S961 increased blood glucose in the insulin treated mice after 2 hours (17.9±4.5 mM STZ-insulin + S961, 4.8±0.5 mM STZ-insulin + vehicle, $P<0.05$) however this dramatic elevation was not observed in the leptin treated group (7.7±2.7 mM STZ-leptin + S961, 4.7±1.1 mM STZ-leptin + vehicle). Similarly, after an oral glucose load the difference in area under the curve was substantially larger in the insulin treated mice (3592±206 STZ-insulin + S961, 737±92 STZ-insulin + vehicle, $P<0.05$) than the leptin treated mice (2530±354 STZ-leptin + S961, 2042±337 STZ-leptin + vehicle). In our complementary model the majority of vehicle treated InsKO mice reached humane endpoint in less than 24 hours after ceasing insulin treatment; however, leptin treatment in InsKO mice prolonged survival for up to 3 weeks. On day 11 of leptin treatment fed blood glucose levels were dramatically elevated in leptin treated InsKO mice compared to Het controls (>33.3 mM vs 10.5±0.7 mM) yet upon 6 hour fasting leptin treated InsKO mice reached dangerously low blood glucose levels compared to Het controls (2.2±0.1 mM vs 4.0±0.3 mM, $P<0.05$), and hyperglycaemia rapidly returned upon 1 hour of re-feeding.

Conclusion: Insulin receptor antagonism and congenital insulin deficiency do not prevent leptin-mediated normalization of fasting glycaemia or survival, suggesting that leptin can act independently of insulin to improve diabetic symptoms in type 1 diabetes.

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Importance of ceramide transporter CERT in the development of muscle insulin resistance

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Background and aims: Accumulation of fatty acids (FA) in muscle cells, a situation that can be observed in obesity, is accompanied by a decrease of insulin sensitivity. FA are metabolized into a lipid derivative called ceramide and studies show that accumulation of ceramide in cells plays a central role in the development of muscle insulin resistance. In physiological condition, ceramide is produced at the endoplasmic reticulum (ER), and transported from the ER to the Golgi, where it is converted to sphingomyelin. The ceramide transporter CERT has been identified as a key factor for the ER-to-Golgi trafficking of ceramide. We tested the hypothesis that a reduction of the activity/expression of CERT could contribute to the accumulation of ceramide in the ER and in the development of insulin resistance in muscle cells.

Materials and methods: We quantified expression of CERT in various insulin resistance models (i) in vitro in C2C12 and human myotubes treated with palmitate and in diabetic patient myotubes and (ii) in vivo in muscle from ob/ob mice as well as mice fed a high fat diet. To figure out whether CERT plays an important role in maintaining insulin sensitivity in muscle cells, we inhibited its expression using siRNA or a CERT chemical inhibitor (HPA-12).

Results: CERT protein expression was decreased in all insulin resistance models. We demonstrated that the decrease in CERT expression observed in the presence of FA excess was not related to the activation of ER stress, action of ceramide produced from palmitate itself or degradation via the proteasome, but through caspase cleavage. In addition, we also showed a protein kinase D dependent hyperphosphorylation on serine residue of CERT in response to palmitate, indicating a decrease of its activity. We further confirmed the involvement of a reduction in CERT expression on insulin sensitivity in muscle cells after either artificially inhibiting CERT expression with a siRNA or inhibiting its activity with the HPA-12 inhibitor. In opposite, inhibition of caspase activity prevented ceramide-induced insulin resistance in muscle cells.

Conclusion: Altogether, these results demonstrate the importance of a normal traffic of ceramide from the ER to the Golgi to preserve muscle cell insulin sensitivity and the importance of CERT expression in this process.

Supported by: the SFD

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Myeloid Sirt1 regulation macrophage infiltration and insulin sensitivity in mice fed a high-fat diet

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Background and aims: Inflammation is an important factor in the development of insulin resistance. Sirt1, a class 3 histone/protein deacetylase, has anti-inflammatory functions. Myeloid-specific Sirt1 deletion promotes macrophage infiltration into insulin-sensitive organs and aggravates tissue inflammation. In this study, we investigated how Sirt1 regulates macrophage locomotion in response to metabolic stresses.

Materials and methods: Myeloid-specific Sirt1-deleted mice (mS1KO) and wild type littermates were fed a 60% calorie high-fat diet (HFD) for 16 weeks. Tissue inflammation and metabolic phenotypes were compared. Bone marrow macrophages from wild type or mS1KO mice were used in *in vitro* chemotaxis assays and macrophage polarization studies.

Results: Sirt1 deletion did not alter body weight gain or food intake. However, mS1KO mice fed a HFD exhibited impaired glucose tolerance with reduced insulin secretion and insulin sensitivity. Consistent with these results, mS1KO pancreatic islets displayed decreased mass with profound apoptotic cell damage and increased macrophage infiltration and tissue inflammation. Liver and adipose tissue from mS1KO mice also showed greater accumulation of macrophages and tissue inflammation. *In vitro* results showed that myeloid Sirt1 deletion stimulated proinflammatory M1-like macrophage polarization of bone marrow macrophages and augmented the migration potential of macrophages toward chemoattractants. The latter effect was mediated by increased expression and activation of nuclear factor κ B/focal adhesion kinase and mitogen-activated protein kinase pathways.

Conclusion: Taken together, our results suggested that myeloid Sirt1 is crucial for macrophage migration and the development of HFD-induced inflammation and metabolic derangements.

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The effects of obesity and insulin resistance on serum NMR metabolic profile during an oral glucose tolerance test: a twin study

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Background and aims: Untargeted metabolic profiling has shown that obesity and insulin resistance (IR) are associated with differences in the levels of many circulating metabolites. Notably, the levels of low molecular weight metabolites (LMWMs) and fatty acids predict future risk of diabetes in healthy individuals. Recent studies have also revealed significant metabolic changes in response to an oral glucose tolerance test (OGTT). Obesity and IR are associated with the magnitudes of these changes, but their relative importances are unknown. In this study, we measured a novel panel of metabolites, including lipoprotein fractions, during an OGTT in healthy young twins discordant for BMI, to control for genetic effects and to compare the effects of acquired obesity and IR on the dynamic metabolic profile.

Materials and methods: 31 rare MZ twin pairs discordant for obesity (Within-pair BMI difference BMI >3kg/m²), along with 119 MZ and DZ control pairs, were identified from cohorts of 22–32-year-old Finnish twins. Their serum was analyzed with Nuclear Magnetic Resonance (NMR) at 4 timepoints (0/30/60/120min) during an OGTT. Based on a 50 percentile cut point for the difference of HOMA index within the discordant pairs, the twins were divided into two groups, indicating whether or not the heavier twins' acquired obesity was accompanied by IR. This allowed analyses of metabolite profiles during the OGTT in unhealthy and healthy obese co-twins as compared with their lean twin pair members.

Results: Glucose ingestion resulted in a significant alteration in the levels of 12 out of 51 measured lipoprotein subclasses, 7 out of 24 lipids and 17 out of 21 LMWMs (Multiple-corrected $P<0.05$). There was a late fall in VLDL triglyceride content, most evident in larger fractions (-0.14 SD units, $P=8.5e-12$). The diameter of VLDL particles decreased (-0.34 SD units, $P=6.2e-06$) and

that of LDL particles increased (0.12 SD units, $P=0.008$). Cholesterol content in very large HDL particles decreased (-0.36 SD units, $P=3.12 \times 10^{-6}$) but rose in medium-sized particles (0.19 SD units, $P=0.016$). Healthy obese MZ co-twins (without IR) had lower HDL cholesterol levels throughout the OGTT, especially in large HDL and HDL2 ($P<3.8 \times 10^{-4}$). In contrast, unhealthy obese co-twins (with IR) showed changes in 32 out of 96 metabolites (corrected $P<0.05$), including lipoproteins, fatty acids and LMWMs. These include three recently discovered risk markers for all-cause mortality: α -acid glycoprotein, citrate and VLDL size.

Conclusion: Ingestion of pure glucose results in considerable change in the lipoprotein, lipid and LMWM profiles. Some lipoprotein changes can be explained by increased hepatic synthesis, but others may be indicative of novel actions of insulin. Acquired obesity primarily affects HDL composition, whereas an accompanied insulin resistance results in widespread changes in the metabolic profile both in the fasting state and during a glucose challenge. *Supported by: AOF, NNF, HUHRE, DRF, FFCR*

OP 07 GLP-1 analogues: clinical efficacy

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Efficacy and safety of liraglutide vs placebo when added to basal insulin analogues in subjects with type 2 diabetes (LIRA-ADD2BASAL): a randomised, placebo-controlled trial

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Background and aims: This trial aimed to establish the superior efficacy and acceptable safety of liraglutide (LIRA) vs. placebo (PLAC) added to pre-existing basal insulin analogue \pm metformin in subjects with inadequately controlled type 2 diabetes.

Materials and methods: Subjects with type 2 diabetes, age 18–80 years, BMI 20–45 kg/m², HbA1c 7.0–10.0% and on stable insulin analogue dose ≥ 20 U/day \pm stable metformin ≥ 1500 mg/day were eligible for participation. In a multi-centre, multi-national, double-blind, parallel-group design, subjects were randomised 1:1 to receive once daily LIRA 1.8 mg or PLAC added to pre-existing treatment for 26 weeks. Following randomisation, insulin adjustments above the pre-trial dose were not allowed. The primary endpoint was the change in HbA1c from baseline to Week 26.

Results: A total of 451 subjects were randomised (226 LIRA; 225 PLAC). All subjects but 1 (LIRA, not exposed) were included in the analysis. Mean baseline characteristics were similar between the two groups (LIRA;PLAC): HbA1c 8.2;8.3%, BMI 32.3;32.2 kg/m², diabetes duration 12.1 years and insulin dose 48.3;45.9 U (geometric mean 40.5 U for both groups). After 26 weeks of treatment, subjects taking LIRA had a greater decrease in HbA1c from baseline than PLAC, and more LIRA subjects reached HbA1c $<7.0\%$ and HbA1c $\leq 6.5\%$ using a lower mean estimated daily dose of basal insulin analogue compared to PLAC (35.8 U vs. 40.0 U; see Table). Subjects taking LIRA also achieved greater decreases from baseline in fasting plasma glucose (FPG), incremental post-prandial self-measured plasma glucose (SMPG), body weight, systolic blood pressure and lipids. Nausea and vomiting occurred more frequently with LIRA than PLAC (22.2% vs. 3.1% and 8.9% vs. 0.9%, respectively). Minor hypoglycaemia (plasma glucose <3.1 mmol/L) occurred in 18.2% and 12.4% of LIRA and PLAC subjects, respectively. No severe hypoglycaemic events (requiring assistance of another person to actively administer resuscitative actions) were reported during this trial.

Conclusion: The addition of LIRA to basal insulin analogues ± metformin significantly improved glycaemic control, which can be attributed to the effect of LIRA on both FPG and post-prandial glucose levels. Additionally, LIRA induced greater weight loss and a reduction in systolic blood pressure and selected lipids compared to PLAC. Typical gastrointestinal symptoms and minor hypoglycaemia were more frequent with LIRA than PLAC. No severe hypoglycaemic events were reported during this trial.

	LIRA (n=225)	PLAC (n=225)	Estimated treatment difference (LIRA – PLAC) or ratio (LIRA/PLAC) [95% CI]	Estimated odds ratio [95% CI]	P-value
HbA _{1c} , % ^{a,b}	-1.30	-0.11	-1.19 [-1.39; -0.99]		<0.0001
Subjects achieving HbA _{1c} <7% by Week 26, % ^c	59.2	14.0		8.91 [5.45; 14.59]	<0.0001
Subjects achieving HbA _{1c} ≤6.5% by Week 26, % ^c	42.9	3.6		20.12 [9.92; 40.84]	<0.0001
Subjects achieving HbA _{1c} <7% with no weight gain and no hypoglycaemia by Week 26, % ^c	41.5	8.6		7.50 [4.36; 12.92]	<0.0001
Fasting plasma glucose, mmol/L ^{a,b}	-1.44	-0.16	-1.28 [-1.70; -0.86]		<0.0001
Post-prandial increments of 7- point SPMG profile, mmol/L ^{a,b}	-0.94	-0.37	-0.57 [-0.94; -0.20]		0.0026
Weight, kg ^{a,b}	-3.54	-0.42	-3.11 [-3.85; -2.37]		<0.0001
Systolic blood pressure, mmHg ^{a,b}	-5.78	-0.76	-5.02 [-7.45; -2.59]		<0.0001
Pulse, beats/min ^{a,b}	3.20	-1.31	4.51 [2.59; 6.43]		<0.0001
Total cholesterol, mmol/L ^{a,c,d}	0.92	0.99	0.93 [0.89; 0.96]		0.0002
LDL cholesterol, mmol/L ^{a,c,d}	0.90	1.00	0.91 [0.86; 0.96]		0.0013
VLDL cholesterol, mmol/L ^{a,c,d}	0.89	0.99	0.91 [0.84; 0.97]		0.0079
HDL cholesterol, mmol/L ^{a,c,d}	0.97	0.99	0.98 [0.95; 1.01]		0.1619
Free fatty acids, mmol/L ^{a,c,d}	1.02	0.99	1.03 [0.95; 1.12]		0.4491
Triglycerides, mmol/L ^{a,c,d}	0.90	0.99	0.90 [0.83; 0.97]		0.0089
Basal insulin analogue dose, U ^{a,c,d}	0.87	0.98	0.89 [0.87; 0.92]		<0.0001

^a Estimated means and p-values from a mixed model for repeated measurements

^b Values represent differences from Week 0 to Week 26

^c Estimated proportions and p-values from a logistic regression analysis

^d Values represent ratios between Week 26 to Week 0

^e Log-transformed prior to analysis

Clinical Trial Registration Number: NCT01617434

Supported by: Novo Nordisk

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Efficacy and safety of once weekly dulaglutide vs insulin glargine in combination with metformin and glimepiride in type 2 diabetes patients (AWARD-2)

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Background and aims: This Phase 3, 78-week, parallel-arm, open-label (double-blinded to dulaglutide [DU] doses) study compared two doses of the once weekly GLP-1 receptor agonist DU with insulin glargine titrated to fasting glucose target, in patients with type 2 diabetes inadequately controlled with maximally tolerated doses of metformin and glimepiride. Metformin and glimepiride were to be continued throughout the study.

Materials and methods: Patients (N = 807; mean baseline characteristics: age 57 years; duration of diabetes 9.1 years; HbA_{1c} 8.1%; body weight 86.3 kg; BMI 31.6 kg/m²) were randomised (1:1:1) to once weekly DU 1.5 mg, DU 0.75 mg, or once daily insulin glargine. The primary objective was to demonstrate DU 1.5 mg was noninferior (margin 0.4%) to insulin glargine for HbA_{1c} change from baseline at 52 weeks. Additional analyses were carried out at 52 and 78 weeks.

Results: At 52 weeks, DU 1.5 mg was superior and DU 0.75 mg was noninferior to insulin glargine on HbA_{1c} change from baseline. The mean insulin glargine dose was 29.4 U. Body weight decreased with both DU doses and increased with insulin glargine. Over the 52-week period, the mean rate of documented symptomatic hypoglycaemia (≤3.9 mmol/L) was 2.0, 2.0, and 3.3 events/patient/year for DU 1.5 mg, DU 0.75 mg, and insulin glargine, respectively. At 78 weeks, HbA_{1c}, body weight, and hypoglycaemia results were similar to 52 weeks, and the mean insulin glargine dose was 31.4 U. Through 78 weeks, four events of severe hypoglycaemia occurred: 2 in patients treated with DU 1.5 mg and 2 in patients treated with insulin glargine. Nausea and diarrhoea were more common with DU 1.5 mg (15.4% and 10.6%) and DU 0.75 mg (7.7% and 9.2%) versus insulin glargine (1.5% and 5.7%) through 78 weeks.

Conclusion: DU 1.5 mg demonstrated superior and DU 0.75 mg noninferior glycaemic control compared with insulin glargine, and this was associated with weight loss, reduced incidence of hypoglycaemia, and an acceptable safety profile.

Table 1. Glycaemic and Body Weight Measures at 52 and 78 Weeks			
Primary Time Point (52 wk, ITT, LOCF)	DU 1.5 mg (N=273)	DU 0.75 mg (N=272)	Insulin Glargine (N=262)
HbA _{1c} change (%), LS Mean (SE)	-1.08 (0.06) ^{††}	-0.76 (0.06) [†]	-0.63 (0.06)
% patients with HbA _{1c} <7.0%	53.2 [#]	37.1	30.9
Fasting serum glucose change (mmol/L), LS Mean (SE)	-1.50 (0.14)	-0.87 (0.14) [#]	-1.76 (0.14)
Body weight change (kg), LS Mean (SE)	-1.87 (0.24) [#]	-1.33 (0.24) [#]	1.44 (0.24)
Final Time Point (78 wk, ITT, LOCF)			
HbA _{1c} change (%), LS Mean (SE)	-0.90 (0.07) ^{††}	-0.62 (0.07) [†]	-0.59 (0.07)
% patients with HbA _{1c} <7.0%	49.0 [#]	34.1	30.5
Fasting serum glucose change (mmol/L), LS Mean (SE)	-1.10 (0.15) [#]	-0.58 (0.16) [#]	-1.58 (0.15)
Body weight change (kg), LS Mean (SE)	-1.96 (0.26) [#]	-1.54 (0.26) [#]	1.28 (0.26)

^{††} multiplicity adjusted 1-sided p <0.001 for noninferiority or superiority vs insulin glargine, respectively, for HbA_{1c} change only

[#] 2-sided p <0.05 vs insulin glargine

Clinical Trial Registration Number: NCT01075282

Supported by: Eli Lilly and Company

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Liraglutide 3.0 mg for weight management in obese/overweight adults with type 2 diabetes: SCALE diabetes 56-week randomised, double-blind, placebo-controlled trial

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Background and aims: Liraglutide at doses up to 1.8 mg is approved for the treatment of T2D. This study investigated the efficacy and safety of liraglutide 3.0 mg and 1.8 mg, as adjunct to diet and exercise, for weight management in obese/overweight adults with T2D.

Materials and methods: In this 56-week, randomised, double-blind, placebo-controlled trial, adults with T2D (on diet and exercise alone or with 1-3 oral antidiabetic drugs [OADs], HbA_{1c} 7-10%, BMI ≥27.0 kg/m²) were randomised 2:1:1 to receive liraglutide 3.0 mg, 1.8 mg or placebo. All subjects received diet (500 kcal/day deficit) and exercise instruction.

Results: 846 individuals were randomised: age 54.9 (18.0-82.0) years, 50% male, BMI 37.1 (27.0-67.6) kg/m², HbA_{1c} 7.9% (6.4-10.3%), fasting plasma glucose (FPG) 8.8 (4.2-17.3) mmol/L, T2D for 7.3 (0.2-36.5) years, 11.5% on diet and exercise, 57.3% on metformin only, 31.2% on combination OADs. Liraglutide 3.0 mg and 1.8 mg were superior to placebo, and 3.0 mg was superior to 1.8 mg on mean and categorical weight loss at week 56 (Table). Liraglutide 3.0 mg also achieved superior glycaemic control vs. placebo and liraglutide 1.8 mg (change in HbA_{1c} and FPG, proportion reaching HbA_{1c} ≤6.5%, and [vs. placebo only] postprandial plasma glucose [PG] increment; Table). The safety profiles with liraglutide 3.0 mg and 1.8 mg were similar, although gastrointestinal disorders were more frequent with 3.0 mg (65% of individuals) than 1.8 mg (56%) and placebo (39%). No cases of pancreatitis were reported during the trial. An increase in mean serum lipase activity was seen with liraglutide 1.8 mg and 3.0 mg; the increase was not dose-dependent and few individuals (7.7% and 9.8% on liraglutide 1.8 mg and 3.0 mg, respectively, vs. 6.3% on placebo) had levels ≥3 times the upper normal range at any time during treatment. Rates of documented symptomatic hypoglycaemia (PG <3.9 mmol/L) were 0.87, 0.95 and 0.31 events per patient year for liraglutide 3.0 mg, 1.8 mg and placebo, respectively; in all groups, hypoglycaemia rates were greater for subjects on a sulphonylurea (SU) compared with those not on SU. Eight severe hypoglycaemic events were reported (5 events in 3 subjects [0.7%] with liraglutide 3.0 mg; 3 events in 2 subjects [1.0%] with liraglutide 1.8 mg); all subjects affected were receiving background SU therapy.

Conclusion: Liraglutide 3.0 mg, as adjunct to diet and exercise, was efficacious and well-tolerated for weight management over 56 weeks in obese/overweight individuals with T2D.

Table 1. Glycaemic and Body Weight Measures at 52 and 78 Weeks			
Primary Time Point (52 wk, ITT, LOCF)	DU 1.5 mg (N=273)	DU 0.75 mg (N=272)	Insulin Glargine (N=262)
HbA _{1c} change (%), LS Mean (SE)	-1.08 (0.06) ^{††}	-0.76 (0.06) [†]	-0.63 (0.06)
% patients with HbA _{1c} <7.0%	53.2 [*]	37.1	30.9
Fasting serum glucose change (mmol/L), LS Mean (SE)	-1.50 (0.14)	-0.87 (0.14) [*]	-1.76 (0.14)
Body weight change (kg), LS Mean (SE)	-1.87 (0.24) [*]	-1.33 (0.24) [*]	1.44 (0.24)
Final Time Point (78 wk, ITT, LOCF)			
HbA _{1c} change (%), LS Mean (SE)	-0.90 (0.07) ^{††}	-0.62 (0.07) [†]	-0.59 (0.07)
% patients with HbA _{1c} <7.0%	49.0 [*]	34.1	30.5
Fasting serum glucose change (mmol/L), LS Mean (SE)	-1.10 (0.15) [*]	-0.58 (0.16) [*]	-1.58 (0.15)
Body weight change (kg), LS Mean (SE)	-1.96 (0.26) [*]	-1.54 (0.26) [*]	1.28 (0.26)

^{†,††} multiplicity adjusted 1-sided p <0.001 for noninferiority or superiority vs insulin glargine, respectively, for HbA_{1c} change only
^{*} 2-sided p <0.05 vs insulin glargine

Clinical Trial Registration Number: NCT01272232

Supported by: Novo Nordisk

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Efficacy and safety of once weekly dulaglutide versus once daily liraglutide in type 2 diabetes (AWARD6)

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Background and aims: This Phase 3, randomised, open-label, parallel-arm 26-week (wk) study compared the efficacy and safety of once weekly dulaglutide (DU) 1.5 mg, a long-acting GLP-1 receptor agonist, with once daily liraglutide (LIRA) 1.8 mg in metformin-treated (≥1500 mg) patients with type 2 diabetes.

Materials and methods: Patients (N=599; mean baseline age, 57 years; HbA_{1c} 8.1 %; weight 94.1 kg) were randomised to DU 1.5 mg or LIRA 1.8 mg in a 1:1 ratio. The primary objective was HbA_{1c} change from baseline at 26 wks tested for noninferiority (margin 0.4%); DU 1.5 mg vs LIRA 1.8 mg.

Results: DU 1.5 mg was noninferior to LIRA 1.8 mg at 26 wks as measured by HbA_{1c} change from baseline (between-group HbA_{1c} change -0.06; 95% CI [-0.19, 0.07]) (Table 1). While both groups experienced significant weight reduction, LIRA-treated patients demonstrated a 0.71 kg greater weight reduction than DU-treated patients (p=0.01). The most common treatment-emergent gastrointestinal adverse events for DU 1.5 mg and LIRA 1.8 mg, respectively, were nausea (20.4%, 18.0%), diarrhoea (12.0%, 12.0%), dyspepsia (8.0%, 6.0%), and vomiting (7.0%, 8.3%). Patients who discontinued study and/or study drug due to gastrointestinal adverse events were similar (DU 1.5 mg [3.0%], LIRA 1.8 mg [4.3%]). Rates of hypoglycaemia (≤3.9 mmol/L ± symptoms) were 0.34 events/pt/yr (DU 1.5 mg) and 0.52 (LIRA 1.8 mg) events/pt/yr. No severe hypoglycaemia was reported.

Conclusion: Once weekly DU 1.5 mg demonstrated noninferior glycaemic control compared to once daily LIRA 1.8 mg with a similar safety and tolerability profile.

Efficacy Measures (26 wk, ITT)	DU 1.5 mg (N=299)	LIRA 1.8 mg (N=300)
HbA _{1c} change, %, Least Square Mean (SE) ^a	-1.42 (0.05) [‡]	-1.36 (0.05)
% of patients with A1C <7.0%	68.3	67.9
Weight change, kg, Least Square Mean (SE) ^b	-2.90 (0.22) [‡]	-3.61 (0.22)

[‡] 1-sided p <0.001 for noninferiority vs LIRA for HbA_{1c} change. ^ap = 0.01 vs LIRA.

^aMMRM. ^bANCOVA LOCF.

Clinical Trial Registration Number: NCT01624259

Supported by: Eli Lilly and Company

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Harmony 1 year 3 Results: albiglutide vs placebo in patients with type 2 diabetes mellitus not controlled on pioglitazone (pio) ± metformin (met)

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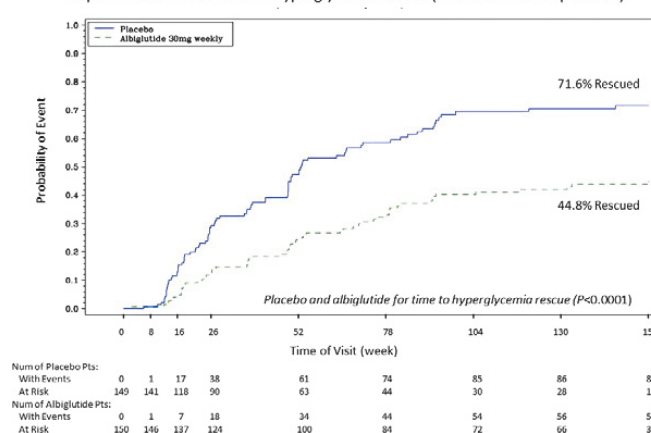
Background and aims: This 3 year (y), randomized, double-blind, placebo (Pbo) controlled study evaluated efficacy & safety of once weekly GLP-1 receptor agonist albiglutide 30 mg vs Pbo in patients (pts) inadequately controlled (A1c 53-85.8 mmol/mol [7-10%]) on Pio ± Met.

Materials and methods: Pts could continue if hyperglycaemic rescue was needed. Primary endpoint (PE) was A1c change from baseline at week 52.

Results: Baseline demographics were similar between groups; mean A1c 65 mmol/mol (8.1%); age 55 y; 80% on Pio + Met. PE showed Albi superior to Pbo (treatment difference: -8.2 mmol/mol [-0.75%], P<0.0001). Glycaemic reduction with Albi was maintained for 3 y: fewer Albi pts required rescue compared to Pbo pts: 45% vs 72%; P<0.0001. In pts completing 3 y of treatment, A1c reduction was durable to wk 156 without rescue (n=54/26 for Albi/Pbo: -9.5 mmol/mol [-0.87%] Albi, -5.5 mmol/mol [-0.50%] Pbo) and with rescue n=89/83, -9.8 mmol/mol [-0.90%] Albi, -3.0 mmol/mol [-0.27%] Pbo). FPG changes were similar to A1c. Change in weight at 3 y was -0.16kg Albi and 1.50kg Pbo. Through wk 156, adverse events (% pts) of nausea and vomiting were low and comparable between Albi/Pbo (12.0%/11.9% and 5.3%/4.0%) while diarrhea was higher with Albi (14.7% vs 10.6%). Injection site reactions were higher for Albi (18.0%) vs Pbo (8.6%). Incidence of pre-rescue documented symptomatic (≤3.88 mmol/L [70 mg/dL]) and severe hypoglycaemia was low: 3.3%/1.3% for Albi and 2.0%/0% for Pbo.

Conclusion: Albi combination therapy resulted in durable glycaemic improvement with good tolerability.

Kaplan-Meier Plot of Time to Hyperglycemia Rescue (Intent-to-Treat Population)



Clinical Trial Registration Number: NCT00849056

Supported by: Research funded by GSK

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Better glycaemic control and less weight gain with once weekly dulaglutide vs bedtime insulin glargine, both combined with thrice daily lispro, in type 2 diabetes (AWARD-4)

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Background and aims: This 52 week, parallel-arm, open-label, phase 3 study compared two doses of the once weekly GLP-1 receptor agonist dulaglutide (DU) versus bedtime insulin glargine, all combined with pre-meal insulin lispro with or without metformin, in patients with type 2 diabetes mellitus inadequately controlled on conventional insulin therapy. Insulin glargine and insulin lispro were titrated to attempt to reach glycaemic targets.

Materials and methods: Patients (N = 884; mean baseline characteristics: age 59.4 years; duration of diabetes 12.7 years; HbA_{1c} 8.5%; body weight 91.1 kg; BMI 32.5 kg/m²; total daily insulin dose 56 U) were randomised (1:1:1) to once weekly DU 1.5 mg, DU 0.75 mg, or bedtime insulin glargine titrated-to-target. The primary objective was to compare the change in HbA_{1c} from baseline of DU 1.5 mg with insulin glargine at 26 weeks for noninferiority (margin 0.4%) and if met, then superiority was tested.

Results: At 26 and 52 weeks, both DU doses were statistically superior to insulin glargine for HbA_{1c} change from baseline. Insulin glargine was associated with greater fasting serum glucose reduction compared with both DU doses. The mean prandial insulin doses at 26 weeks were 93 U for DU 1.5 mg, 97 U for DU 0.75 mg, and 68 U for insulin glargine. The insulin glargine dose was 65 U. Similar insulin doses were observed at 52 weeks. Body weight decreased with DU 1.5 mg and increased with DU 0.75 mg and insulin glargine at 52 weeks. The rate of documented symptomatic hypoglycaemia (≤ 3.9 mmol/L) at 52 weeks was 31.0, 35.0, and 39.9 events/patient/year for DU 1.5 mg, DU 0.75 mg, and insulin glargine, respectively. The number of severe hypoglycaemia events was 11 for DU 1.5 mg, 15 for DU 0.75 mg, and 22 for insulin glargine. Nausea, diarrhoea, and vomiting were more common with DU 1.5 mg (25.8%, 16.6%, and 12.2%, respectively) and DU 0.75 mg (17.7%, 15.7%, and 10.6%) versus insulin glargine (3.4%, 6.1%, and 1.7%).

Conclusion: DU compared to insulin glargine, both combined with insulin lispro, resulted in better glycaemic control, less body weight gain, no increased risk of hypoglycaemia, and more common reporting of gastrointestinal adverse events.

Table 1. Glycaemic and Body Weight Measures at 26 and 52 Weeks			
Primary Time Point (26 wk, ITT, LOCF)	DU 1.5 mg N=295	DU 0.75 mg N=293	Insulin Glargine N=296
HbA _{1c} change (%), LS Mean (SE)	-1.64 (0.07) ^{††}	-1.59 (0.07) ^{††}	-1.41 (0.07)
% of patients with HbA _{1c} <7.0%	67.6*	69.0*	56.8
Fasting serum glucose change (mmol/L), LS Mean (SE)	-0.27 (0.20)*	0.22 (0.20)*	-1.58 (0.20)
Weight change (kg), LS Mean (SE)	-0.87 (0.27)*	0.18 (0.27)*	2.33 (0.27)
Final Time Point (52 wk, ITT, LOCF)			
HbA _{1c} change (%), LS Mean (SE)	-1.48 (0.08) ^{††}	-1.42 (0.08) ^{††}	-1.23 (0.08)
% of patients with HbA _{1c} <7.0%	58.5*	56.3	49.3
Fasting serum glucose change (mmol/L), LS Mean (SE)	0.08 (0.22)*	0.41 (0.22)*	-1.01 (0.22)
Weight change (kg), LS Mean (SE)	-0.35 (0.34)*	0.86 (0.33)*	2.89 (0.33)

^{††} multiplicity adjusted 1-sided p < 0.025 for superiority vs insulin glargine for HbA_{1c} change only
* 2-sided p < 0.05 vs insulin glargine

Clinical Trial Registration Number: NCT01191268

Supported by: Eli Lilly and Company

OP 08 Matters of the heart

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Does diabetes affect the efficacy of dual antiplatelet therapy in patients with acute coronary syndromes?

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Background and aims: Diabetes is a strong, independent risk factor of acute coronary syndromes (ACS). Diabetes aggravates the course of ACS and increases the risk of its complications. Recently, there is growing amount of data about failure in antiplatelet response, which is specifically associated with insulin resistance and diabetes. This incomplete antiplatelet response may contribute to a worse prognosis of ACS in diabetic patients. The aim of this study was to clarify the impact of presence of diabetes on the efficacy of dual antiplatelet therapy given in standard doses in patients with ACS.

Materials and methods: 82 patients with ACS (53 men, 29 women, mean age 65 years) were enrolled in this preliminary prospective observational study. Patients were treated with aspirin loading dose (400 mg) and ADP receptor antagonist loading dose: in 62 patients clopidogrel (600 mg) and in 20 patients prasugrel (60 mg) was used. 21 patients had diabetes. Coronary angiography and percutaneous coronary intervention of culprit coronary lesion was subsequently performed. Light transmission aggregometry (LTA) with specific inducers and VASP phosphorylation assessment was chosen for antiplatelet therapy efficacy testing. Samples were taken after first maintenance dose administration (sample 1) and on 30th day from loading dose administration (sample2).

Results: Mean LTA measured platelet reactivity was 32.7±18.9% in sample1 and 28.4±14.4% in sample2 respectively. No significant difference in antiplatelet response on ADP receptor antagonist (ADP-RA) between diabetic and non-diabetic patients was found (sample1: 30.7±19.8% versus 33.5±18.8%; sample2: 28.4±12.1% versus 28.4±15.7%). Totally 38 non-responders on ADP-RA were identified; ADP-RA unresponsiveness was not associated with the presence of diabetes. Prasugrel therapy generally showed better platelet inhibition than clopidogrel administration (sample1: 22.8±13.7% versus 35.8±19.3%, p<0.01; sample2: 20.0±9.9% versus 33.1±14.6%, p<0.05).

Conclusion: ADP-RA therapy unresponsiveness was frequently (46.3%) identified in ACS patients undergoing coronary angiography. However, diabetes was not connected with higher platelet reactivity or higher prevalence of ADP-RA non-response in this study. Prasugrel showed significantly better platelet inhibition than clopidogrel and thus prasugrel therapy should be preferred not only in diabetic ACS patients. Laboratory monitoring of antiplatelet therapy efficacy may help identify patients with inadequate antiplatelet response.

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Impact of diabetes mellitus on long-term prognosis in patients with ischaemic heart failure: a report from the Swedish Heart Failure Registry (S-HFR)

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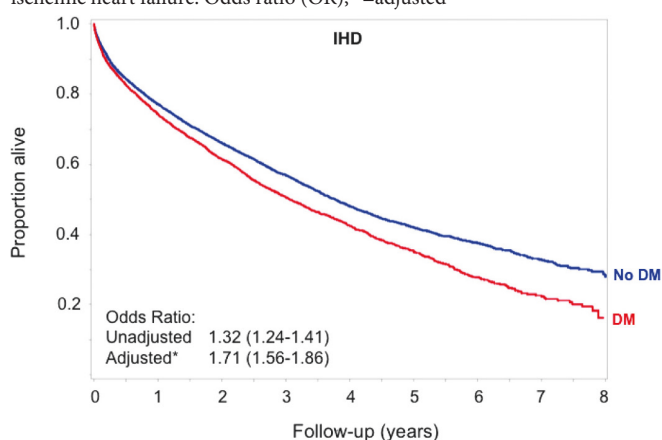
Background and aims: Patients with diabetes are at increased risk of developing heart failure. In an everyday life setting, we investigated the impact of diabetes on long-term prognosis in patients with heart failure of ischemic origin.

Materials and methods: Patients clinically judged to have ischemic heart failure, of whom 50% were previously revascularised with (n=5265) and without (n=12 408) type 2 diabetes, included in the Swedish Heart Failure Registry (S-HFR) 2003-2011 were followed for mortality until 30 September

2011 (median 22.5 months). Differences in background characteristics were adjusted for in a logistic regression model.

Results: Patients with diabetes were younger (75 vs. 77 years) and more often had preserved renal function (>60 ml/min; 44 vs. 38%), however hypertension was more common in those with diabetes (59 vs. 45%). EF did not differ, 17% in both groups had an EF \geq 50%, however those with diabetes had more of severe heart failure symptoms (NYHA III-IV; 53 vs. 46%). Among those with diabetes, 88% received beta-blockade, 61% ACE inhibitors, 67% Statins and 71% Aspirin. Kaplan-Meier curves of mortality are presented in Figure 1. The unadjusted and adjusted* ORs (95% CI) for mortality were 1.32 (1.24–1.41) and 1.71 (1.56–1.86). In those revascularised (50%), unadjusted and adjusted* ORs for mortality were 1.52 (1.39–1.67) and 1.63 (1.45–1.84).

Conclusion: Diabetes is an independent predictor of long-term mortality in patients with ischemic heart failure. Revascularisation did not abolish the impact of diabetes. The use of anti-ischemic secondary preventive treatment seemed somewhat low considering all patients were classified to have ischemic heart disease * Adjusted for gender, age, duration of heart failure, weight, blood pressure, hypertension, atrial fibrillation, pulmonary disease, EF class, revascularisation, eGFR class, Hb class and pharmacological treatment. Figure 1. Kaplan-Meier curve showing long-term survival by diabetes (DM) in patients with ischemic heart failure. Odds ratio (OR), *=adjusted



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Impact of diabetes mellitus on long-term prognosis in patients with preserved heart failure: a report from the Swedish Heart Failure Registry (S-HFR)

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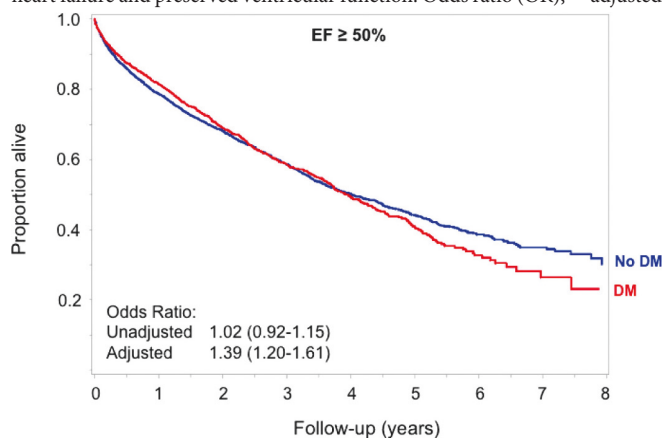
Background and aims: Patients with diabetes are at increased risk for developing heart failure. We investigated the impact of diabetes on long-term prognosis in patients with heart failure and preserved left ventricular function from an everyday life perspective.

Materials and methods: Patients with EF \geq 50%, with (n=1658) and without (n=5047) type 2 diabetes included in the Swedish Heart Failure Registry (S-HFR) 2003–2011 were followed for mortality until 30 September 2011 (median 22.5 months). Differences in background characteristics were adjusted for in a logistic regression model.

Results: Patients with diabetes were younger (76 vs. 78 years), more often had known ischemic heart disease (47 vs. 36%), hypertension (68 vs. 52%), and more often had preserved renal function (eGFR > 60ml/min, 45 vs. 38%). NYHA classes III and IV were somewhat more common in those with diabetes (44 vs. 39%). Kaplan-Meier curves for mortality are presented in Figure 1. The unadjusted and adjusted* ORs (95% CI) for mortality were 1.02 (0.92–1.15) and 1.39 (1.20–1.61).

Conclusion: Our data support that diabetes is an independent predictor of mortality in patients with heart failure and preserved left ventricular function. As much as 50% of patients with preserved left ventricular function had

reported ischemic heart disease, which questions the concept of a pure diabetic cardiomyopathy. * Adjusted for gender, age, duration of heart failure, weight, blood pressure (systolic and diastolic), ischemic heart disease, hypertension, atrial fibrillation, pulmonary disease, revascularisation, eGFR class, Hb class, ACE inhibitors, ARBs, beta-blockers, mineralocorticoid receptor antagonists, diuretics, digitalis, nitrates, statins and antithrombotic agents. Figure: Kaplan-Meier curve showing survival by diabetes in patients with heart failure and preserved ventricular function. Odds ratio (OR), *=adjusted



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Methylglyoxal-induced endothelial dysfunction has a role in the development of heart failure in a mouse model of type 1 diabetes

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Background and aims: Diabetes mellitus increases the risk of heart failure independent of coronary artery disease or hypertension. Endothelial dysfunction (ED) and inflammation, both common in diabetes, are partly caused by the accumulation of methylglyoxal (MG), the primary substrate of the enzyme glyoxalase 1 (GLO1). We have generated transgenic mice that carry the human GLO1 transgene under the control of the preproendothelin promoter. Using this mouse model we examined if the over-expression of GLO1 could reduce MG-induced ED and prevent the development of heart failure caused by streptozotocin (STZ)-induced hyperglycaemia.

Materials and methods: GLO1 transgenic mice and their wildtype (WT) littermates were treated with STZ (WT-diabetic and GLO1-diabetic mice) or vehicle (WT-control and GLO1-control mice). GLO1 activity was measured in endothelial cells (ECs) and cardiomyocytes isolated from the hearts. Eight weeks after STZ treatment, blood serum levels of the ED markers E-selectin, ICAM and VCAM were determined by ELISA. Heart function was assessed using echocardiography. Protein expression of the receptor for advanced glycation end products (RAGE) and neurogulin in the heart was determined by western blot. Apoptosis, detected by TUNEL assay, and capillary numbers, expressed by number of CD31+ cells were determined by immunohistochemistry. QPCR for the Bcl-2 pro-survival gene was done on ECs collected from the digested hearts.

Results: Increased GLO1 activity was confirmed to be 5.6-fold greater in aortic endothelial cells (ECs) of GLO1-mice compared to their WT littermates. Also, the over-expression and increased activity of GLO1 in the heart was restricted to ECs, and not observed in cardiomyocytes. Eight weeks post-STZ elevated serum levels of all 3 ED markers was observed in WT-diabetic mice compared to all other groups (E-selectin \geq 1.3-fold, VCAM \geq 1.1-fold, and ICAM \geq 6.7-fold; $p \leq 0.04$). RAGE levels were significantly higher in hearts of WT-diabetic mice compared to all other groups (\geq 1.8-fold, $p \leq 0.03$). The WT-diabetic group had lower left ventricular ejection fraction (EF; 43.6%) and fractional shortening (FS; 28.6%), compared to the other 3 groups (\geq 67.5% (EF) and \geq 42.7% (FS); $p \leq 0.01$). The number of CD31+ ECs in WT-diabetic hearts was reduced by 46% compared to both non-diabetic groups and GLO1-diabetic mice ($p = 0.03$). Apoptotic cells were more numerous in the hearts of WT-diabetic mice (0.44% of cells), compared to non-diabetic WT (0.03%) and GLO1-diabetic mice (0.07%; $p \leq 0.04$). The reduced number of

ECs seen in WT-diabetic mice may also be involved in the 3-fold reduction of neurotrophin, an EC-produced protein that supports cardiomyocyte survival ($p=0.04$). The improved survival of ECs in GLO1-diabetic mice may be related to the preserved mRNA levels of Bcl-2, which can be modified by MG. Bcl-2 was down-regulated 2-fold in WT-diabetic ECs compared to the other groups ($p=0.04$).

Conclusion: Taken together, these results suggest that increased GLO1 activity in ECs reduced ED, cell death and inflammation, and preserved cardiac function, despite high blood glucose levels.

Supported by: Heart and Stroke

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A relationship of asymptomatic coronary artery disease and type 2 diabetes in acute ischaemic stroke patients; cerebral angiography and coronary angiography study

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Background and aims: Stroke and coronary artery disease (CAD) share similar atherosclerosis risk factors such as smoking, hypertension, dyslipidaemia, and diabetes. CAD is considered the leading cause of mortality and morbidity in patients with ischemic stroke. However, data about the prevalence of CAD in the patients with acute ischemic stroke remains limited. Furthermore, characteristics of CAD in patients with diabetes are asymptomatic onset and multiple vessels involvement. But it remains unclear whether the impact of diabetes on CAD is different in ischemic stroke. The aim of this study was to determine the relation of type 2 diabetes and acute ischemic stroke to the presence and extent of asymptomatic CAD by coronary angiography.

Materials and methods: Acute ischemic stroke patients without known CAD were enrolled between October 2008 and September 2013 in our hospital. Patients with high Framingham Risk Score (FRS; a 10 year-risk of coronary heart disease $\geq 20\%$) underwent cerebral angiography and coronary angiography at the same time. We analyzed the coronary angiography and other classic atherosclerosis risk factors in 187 patients retrospectively. The patients were divided into diabetes group and non-diabetes group, and the data was assessed according to the following classifications: (1) presence of coronary artery stenosis, (2) presence of significant stenosis ($\geq 50\%$), (3) presence of multiple coronary artery stenosis (≥ 2 vessels), and (4) presence of significant multiple coronary artery stenosis (≥ 2 vessels). We calculated the prevalence of CAD with 95% confidence intervals. All data was analyzed after adjusting classic atherosclerosis risk factors such as age, sex, hypertension and history of past stroke. P value <0.05 was considered to be statistically significant.

Results: Mean age was 64.5 ± 10.9 years and 126 patients (67.4%) were male. 59 patients (M:F 35:24) had type 2 diabetes, 128 patients (M:F 91:37) had no history of diabetes. A total of 129 patients (69%; 48 with diabetes, 81 without diabetes) had hypertension (p -value < 0.008). Past stroke history was shown in 12 patients with diabetes, 15 patients without diabetes (p -value 0.15). The prevalence of coronary artery stenosis was 93% ($n=55$) in the diabetes group, 82% ($n=105$) in the non-diabetes group (p -value 0.08). 31 patients (52.5%) had significant stenosis in the diabetes group, 49 patients (38.2%) had it in the non-diabetes group (p -value 0.019). Multiple coronary artery stenosis was shown in 42 patients (71%) with diabetes, 72 patients (56%) without diabetes (p -value 0.04). The prevalence of significant multiple stenosis was 28.8% ($n=17$) in the diabetes group, 20.3% ($n=26$) in the non-diabetes group (p -value 0.019). In the diabetes group, the patients had high FRS ($40.9\% \pm 8.59$) and had a tendency of both intracranial and extracranial stenosis in cerebral angiography ($n=29$). The characteristics of coronary artery stenosis did not seem to correlate with the severity or location of cerebral artery stenosis.

Conclusion: This study shows that type 2 diabetes with acute ischemic stroke was strongly associated with asymptomatic and severe coronary artery disease. We suggest that assertive evaluation about asymptomatic CAD might be considered in type 2 diabetic patients with acute ischemic stroke.

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Cardiac ^{82}Rb -PET/CT reveals microvascular dysfunction in asymptomatic patients with type 2 diabetes

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Background and aims: Type 2 diabetes (T2DM) is commonly accompanied by asymptomatic coronary artery disease. Coronary flow reserve (CFR; CFR = stress divided by rest coronary blood flow) and coronary calcium score (CCS) represent different aspects of atherosclerosis. CFR is a measure of vasodilator function and reflects physiologic changes of vascular smooth muscle as well as endothelial function and detects coronary microvascular dysfunction. CCS is an anatomic measure of atherosclerosis. Cardiac ^{82}Rb positron emission tomography (PET)/computed tomography (CT) allows for simultaneous non-invasive assessment of both CFR and CCS. We aimed 1) to gain information of the prevalence and predictors of reduced CFR and high levels of CCS in T2DM patients free of overt CV disease (with or without albuminuria) while comparing them to non-diabetic controls, and 2) to determine the association between CFR and CCS.

Materials and methods: Cross-sectional study of 60 T2DM patients stratified by normoalbuminuria ($<30\text{mg}/24\text{h}$) ($n=30$; age (mean \pm SD) 60.9 ± 10.1 years; 40% women) and albuminuria ($\geq 30\text{mg}/24\text{h}$) ($n=30$; 65.6 ± 4.8 years; 27% women) randomly selected from our outpatient clinic and 30 non-diabetic controls (59.8 ± 9.9 years; 40% women) undergoing quantitative rest and pharmacologic stress ^{82}Rb PET/CT and comprehensive assessment of other cardiovascular risk factors.

Results: In controls, normoalbuminuric, and albuminuric patients the CFR was 3.0 ± 0.8 , 2.6 ± 0.8 , and 2.0 ± 0.5 ($P < 0.001$); frequency of reduced CFR (<2.5) was 16.7, 40.0, and 83.3% ($P < 0.001$); and CCS (median [IQR]) was 0 [0–81], 36 [1–325], and 370 [152–1025] ($P < 0.001$), respectively. After adjustment for age, gender, body mass index, 24-h systolic blood pressure, heart rate, cholesterol, and smoking the level of CFR remained significant higher ($P=0.023$) and CCS significant lower ($P=0.020$) in normoalbuminuric vs. albuminuric patients. Moreover, the frequency of reduced CFR remained significant lower in normoalbuminuric vs. albuminuric patients ($P=0.001$) and in controls vs. albuminuric patients ($P < 0.001$). The univariate association between CFR and CCS was significant in control subjects ($R^2 = 0.23$; $P=0.007$), but not in normoalbuminuric or albuminuric patients ($R^2 \leq 0.10$; $P \geq 0.09$). Multivariate linear regression analysis (including variables as above and additionally urine albumin excretion (UAER) and HbA_{1c}) was used to identify variables independently associated with CFR and CCS in the three groups. Lower CFR was significantly related to higher CCS, UAER, age, heart rate, lower HbA_{1c} , and male gender ($P \leq 0.047$) in albuminuric patients; and to female gender ($P=0.024$) in normoalbuminuric patients. Higher CCS was significantly related to lower CFR and UAER ($P \leq 0.048$) in albuminuric patients; and to higher HbA_{1c} in controls ($P=0.033$).

Conclusion: In T2DM patients free of overt cardiovascular disease the prevalence of coronary microvascular dysfunction was high, especially with concomitant albuminuria. This was not explained by elevated CCS (“atherosclerosis”) and prospective studies are needed to show the prognostic significance.

Supported by: H-3-2013-015

OP 09 Diabetic retinopathy

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Validation of model to estimate risk of progression of diabetic retinopathy using screening and clinical data in 3 cohorts

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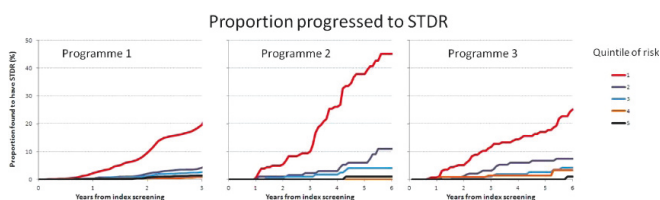
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Background and aims: Diabetic retinopathy (DR) is a microvascular complication of diabetes and can lead to vision loss and blindness. Annual screening with 2 field digital retinal imaging after mydriasis is recommended by the UK National Screening Committee. This is becoming difficult to achieve because of resource limitations and increasing numbers of people with diabetes. We have developed and validated a model to estimate risk of progression to sight threatening DR (STDR) using results from one screening episode, HbA_{1c} in twelve months prior to screening and duration of diabetes in one English screening programme. We know that grading protocols differ between screening programmes and rates of DR are affected by duration of diabetes, ethnicity, deprivation and diabetic control. Here we examine the performance of the model in 3 more English programmes.

Materials and methods: Data were obtained from 3 English screening programmes and data for a subset of patients was extracted from primary care. Patients free of STDR were categorised into those with No DR, those with mild non proliferative DR (NPDR) in one eye and those with Mild NPDR in both eyes. Using the risk estimation algorithm the risk score in those free of STDR was estimated and progression to STDR in quintiles of risk obtained.

Results: The programmes had 8632, 1084 and 1377 people respectively. There were few non White Caucasian patients in the first 2 programmes but 30% of those in the third programme were of African or Afro-Caribbean ethnicity. Duration of diabetes was 6 (2 to 11) (median (25th to 75th centile)), 2.9 (0.6 to 6.2) and 3.6 (1.4 to 6.8) years, HbA_{1c} 56 (48 to 66), 53 (46 to 53) and 52 (45 to 64), follow-up from date of index screening 2.9 (2.1 to 3.0), 4.2 (2.2 to 5.3) and 4.1 (2.1 to 6.9) years. In the first programme the rate of progression to STDR in the lowest risk quintile was 4.1 per 1000 patient years and in highest quintile 74.0 per 1000 patient years, in the second programme 2.4 and 79.2 respectively and in the third programme 1.7 and 49.0 respectively. Area under the ROC curve was 0.82, 0.87, and 0.77.

Conclusion: Within each of the three programmes examined the risk model discriminates well between those with very low and with high risk of progression to STDR. The algorithm would be suitable for calculation of personalised screening intervals. Further validation in other screening programmes and ethnic groups is required.



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Importance of using HbA_{1c} data of total diabetes duration in studying the relation between HbA_{1c} value and retinopathy in type 1 diabetes

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Background and aims: Glycaemic memory, in which past glycaemia could affect future retinopathy, is a potential issue in studying the relation between glycosylated haemoglobin A1c (HbA1c) value and retinopathy. We have

demonstrated that mean HbA1c value (mA1C) covering total diabetes duration, which might reflect the full effect of glycaemic memory, could substantially predict retinopathy, but the longer the period without HbA1c data following diabetes onset in simulation, the less accurate the prediction in our type 1 diabetes mellitus (T1DM) patients. To confirm the importance of using HbA1c data of total diabetes duration, we examined the relation between mA1C and retinopathy in the public data of DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) studies.

Materials and methods: From the primary prevention cohort, we included T1DM patients with the shortest diabetes duration (≤ 12 months) at DCCT baseline. We developed a window which we named 'half-yearly visit (HYV)' to calculate the mA1C and to determine the retinopathy steps of the final Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale for persons. HbA1c values of an even number of quarterly visits (QVs) were applied to those of HYVs: for example, the value of QV00 (DCCT baseline) to HYV00, QV02 to HYV01, and so on during DCCT. During EDIC, HbA1c values were applied to the corresponding HYVs according to their data collection dates from DCCT baseline: for example, 11.75 $\leq X < 12.25$ years' data to HYV24 (12 years from DCCT baseline). Missing values were estimated by time-weighted averaging from the preceding and the succeeding HYV values. An mA1C at HYV24 (mA1C(HYV24)) was calculated by supposing that each patient had a diabetes duration of one year at HYV00 and had kept the same HbA1c value of HYV00 during that period. We also calculated simulated mA1C(24HYV) s by masking HbA1c data for periods of 3, 6, and 9 years following diabetes onset, that is, by averaging HbA1c values from HYV05, HYV11, and HYV17 to HYV24, respectively. Retinopathy was defined as positive if a patient's ETDRS step was 4 or above at HYV24; otherwise it was considered negative. The Wilcoxon rank-sum test was performed. Two-sided $P < 0.05$ was considered significant.

Results: Of the 55 cases who fulfilled the criteria, 40 cases (mean duration 10.9, range 8–12 months) had retinopathy data at HYV24. The 40 values of mA1C(HYV24) were well-divided between the retinopathy-positive and -negative groups ($P = 0.0003$). In simulated cases of mA1C(HYV24) masked during periods of 3, 6, and 9 years, the differences seemed to lessen gradually between the two groups ($P = 0.0009$, 0.0145 and 0.180, respectively), and there was no significant difference in mA1C(HYV24) masked for 9 years. It seemed that the longer the period without HbA1c data following onset in simulation, the less difference between the two groups. This could be explained by the difference of the transitions of the mean HbA1c values in each HYV between the two groups over the course of 12 DCCT/EDIC years. In the earlier period (HYV01 to HYV18), these values were higher in the retinopathy-positive group ($P < 0.05$ for each HYV), but gradually decreased until there was no significant difference between groups (HYV19 to HYV24).

Conclusion: It is important to consider using HbA1c data of total diabetes duration in studying the relation between HbA1c value and retinopathy in T1DM patients.

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Genetic variation of SLC19A3 is associated to diabetic retinopathy and nephropathy in type 1 diabetes

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Background and aims: Diabetes duration, glycaemic control and blood pressure control are the strongest known risk factors for the development of diabetic microangiopathy. Thiamine (Vitamin B1) regulates intracellular glucose management at different levels and has been shown to correct high glucose-induced abnormalities by reducing reactive oxygen species production. Thiamine and its derivative benfotiamine have also been shown to reduce progression of retinopathy and nephropathy in animals with experimental diabetes. According to our hypothesis, patients who are prone to develop diabetic retinopathy and/or nephropathy may have impaired ability to achieve sufficiently high intracellular thiamine levels. This might be particularly damaging in insulin independent tissues that are more exposed to hyperglycaemia because they cannot regulate glucose inflow, such as retinal capillary endothelium and pericytes, the neuroretina, renal podocytes and mesangium. The aim of this study was to test if mutations in thiamine trans-

porters hTHT1 and hTHT2 (coded by *SLC19A2* and *SLC19A3*, respectively), and/or their transcription factors Sp1 and Sp2 (coded by *SP1* and *SP2*), are associated with proliferative diabetic retinopathy (PDR) and/or diabetic nephropathy (DN).

Materials and methods: The patient population consisted of 1568 cases with PDR, and 217 controls with no/mild retinopathy from the Finnish Diabetic Nephropathy (FinnDiane) Study. PDR was defined as ETDRS-score of 53 or worse or scattered laser treatment. Controls were required to have minimal diabetes duration of 20 years, maximal ETDRS-score of 30 on altered ETDRS-scale (corresponding to mild retinopathy) and no laser treatment. We chose HapMapII imputed SNPs from all four candidate genes and 10kb up and downstream of them (n=134). Logistic regression adjusted for sex, age, diabetes duration, and first ten genetic principal components were used for statistical calculations. In addition, association was tested between patient groups with different states of DN, and intersection of classes with both severe PDR and end-stage renal disease (ESRD) (n=369) vs. normo/microalbuminuric controls with no/mild retinopathy (n=190).

Results: Two SNPs, rs12694743 and rs6713116, showed association with PDR (rs12694743: OR = 1.91, CI95% = 1.62–2.19, $P = 1.02 \times 10^{-5}$; rs6713116: OR = 2.34, CI95% = 1.97–2.72, $P = 9.10 \times 10^{-6}$). Both SNPs are located in intronic regions of *SLC19A3*. The signal was also noticeable in DN phenotypes, but was not significant after correcting for multiple testing. In analysis of intersection of extreme phenotypes the signal was amplified (rs12694743: OR = 3.18, CI95% = 2.76–3.60, $P = 7.51 \times 10^{-8}$; rs6713116: OR = 3.87, CI95% = 3.33–4.40, $P = 7.49 \times 10^{-7}$) providing proof of association not only with retinopathy, but both retinopathy and nephropathy. Replications in DCCT/EDIC and WESRD cohorts are ongoing.

Conclusion: The results of this study suggest that mutations in *SLC19A3* may increase susceptibility to PDR and especially to the combined phenotype of PDR and ESRD.

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Microvesicles derived from mesenchymal stem cells in diabetic-like conditions increase permeability in a retinal blood-barrier model

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Background and aims: Loss of pericytes in the early phases of diabetic retinopathy may disrupt their stable association with endothelial cells (EC), leading to EC proliferation and, eventually, angiogenesis. Microvesicles (MV) are small membrane particles derived from different cells which contain biologically active proteins and RNA and are known to promote phenotypic changes in target cells. MV derived from injured cells may induce dedifferentiation of pericytes, allowing their detachment from vessels. We have previously shown that MV derived from mesenchymal stem cells (MSC), but not from EC, induce pericyte detachment and that diabetic-like conditions (high glucose and hypoxia) play a synergistic role in this destabilizing influence. This study aimed at evaluating whether MV produced by MSC in hypoxia and/or high glucose are able to influence the retinal blood-barrier permeability and to explore the possible role of matrix metalloproteases (MMP) in MV-induced pericyte detachment.

Materials and methods: We used commercially available human microvascular EC (HMEC) and MSC from bone marrow, while human retinal pericytes (HRP) had been previously immortalized in our laboratory. A blood-barrier model was established by seeding HMEC on the porous membrane of transwell inserts, letting them adhere for 24 hrs and then adding HRP into the same insert. MV were extracted from the supernatant of MSC cultured in 1) physiological conditions (NG) 2) hypoxia (hypo) 3) high glucose (HG) 4) HG + hypo. These MV were subsequently added to the confluent HMEC/HRP co-cultures. After 2 hrs of MV exposure, FITC was added into the upper chamber and fluorescence measured in the lower chamber of the inserts after another 30', 1, 2, 3, 4 and 24h. MMP expression in both MV and supernatants of HRP exposed to MV was evaluated by Zimography. Results were confirmed by pre-treatment of MV with batimastat, a MMP inhibitor, and subsequent exposure of HRP to them.

Results: Permeability of EC-HRP co-cultures was increased by exposure to MSC-derived MV obtained in all the above conditions, the highest percentage increase occurring after 6h of total exposure to MV (2h pre-treatment + 4h FITC): NG-MV $134.42 \pm 17.46\%$ ($p < 0.05$ vs control without MV), HG-MV $128.07 \pm 3.81\%$ ($p = 0.000$), NG+hypo-MV $119.87 \pm 15.56\%$ ($p < 0.05$), HG+hypo-MV $134.60 \pm 15.21\%$ ($p < 0.05$). MSC-derived MV expressed MMP-2 and MMP-9. The same MMPs were expressed by HRP following 24h exposure to MV, MMP-2 to a much higher degree. HRP number decreased after 4h incubation with untreated MV ($75.81 \pm 9.22\%$, $p < 0.05$ vs control), while pre-incubation of MV with batimastat completely reverted their effect on pericyte detachment.

Conclusion: MSC-derived MV may play a role in vessel destabilization and increase of retinal blood-barrier permeability during the early stages of retinopathy. This effect may be mediated by increased MMP expression.

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Fenofibrate prevents the disruption of the outer blood retina barrier through downregulation of NF- κ B activity

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Background and aims: There is clinical evidence that fenofibrate, a PPAR α agonist, arrests the progression of diabetic macular edema (DME). However, the underlying mechanisms of this beneficial effect remain to be elucidated. We previously reported that fenofibric acid (FA), the active metabolite of fenofibrate, prevents the disorganization of tight junction proteins and the hyperpermeability provoked by the diabetic milieu in the retinal pigment epithelium (RPE). The aim of the present study was to evaluate whether this effect is mediated by inhibiting the proinflammatory transcription factor NF- κ B, as well as the expression of several proinflammatory cytokines involved in the pathogenesis of DME.

Materials and methods: Human RPE were cultured under standard conditions and under conditions leading to the disruption of the monolayer (IL-1 β (10 ng/mL)). The effect of FA, QNZ (a NF- κ B inhibitor), WY14643 (a PPAR α agonist), and MK-886 (a PPAR α antagonist) in preventing the disruption of the monolayer was determined by dextran permeability and immunohistochemistry analyses. The effect of FA on NF- κ B activity was assessed by EMSA and by NF- κ B/p65 nuclear translocation analyses. The expression of cytokines (IL-6, IL-8, MCP-1) was measured by RT-PCR.

Results: FA prevented RPE monolayer disruption, and the consequent hyperpermeability induced by IL-1 β , through inhibition of NF- κ B activity. This effect was due to PPAR α activation and was associated with a significant downregulation of the expression of proinflammatory cytokines.

Conclusion: Our findings suggest that the anti-inflammatory effects of FA through inhibition of NF- κ B activity play a key role in the beneficial effect of fenofibrate for treating DME.

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Diabetic macular oedema and diabetic retinopathy: treatment outcomes with aflibercept do not depend on systemic diabetes control

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Background and aims: Diabetic macular edema (DME) is a major cause of vision loss in patients with diabetic retinopathy resulting from poorly controlled diabetes mellitus. Vascular endothelial growth factor plays a key role in the pathogenesis of DME.

Materials and methods: Two similarly designed Phase 3 trials, VIVID-DME and VISTA-DME, evaluated the efficacy and safety of intravitreal aflibercept (IVT-AFL) injection for the treatment of DME. These trials randomised 872 patients with DME 1:1:1 to receive either IVT-AFL 2 mg every 4 weeks plus sham laser (2q4), IVT-AFL 2 mg every 8 weeks following 5 initial monthly doses plus sham laser (2q8), or macular laser photocoagulation plus sham IVT treatment. The primary efficacy endpoint was the mean change in best-corrected visual acuity (BCVA) from baseline at Week 52. An exploratory analysis examined BCVA and diabetic retinopathy severity score (DRSS) outcomes in subgroups of patients in these studies with baseline haemoglobin A1c (HbA1c) levels $\leq 8\%$ and $> 8\%$.

Results: Overall, 65% and 35% of patients had HbA1c $\leq 8\%$ and $> 8\%$ at baseline, respectively. The mean change in BCVA from baseline to Week 52 in the 2q4 and 2q8 groups versus the laser group was +12.3 and +10.9 versus +1.1 letters ($P < 0.0001$) in patients with baseline HbA1c $\leq 8\%$, and +10.4 and +10.3 versus -0.3 letters ($P < 0.0001$) in patients with baseline HbA1c $> 8\%$, respectively. The proportion of patients in the 2q4 and 2q8 groups versus the laser group with ≥ 2 -step improvement in DRSS score at Week 52 was 34.7% and 29.8% versus 12.3% ($P \leq 0.0001$) in patients with baseline HbA1c $\leq 8\%$, and 33.3% and 26.5% versus 11.4% ($P < 0.03$) in patients with baseline HbA1c $> 8\%$, respectively. The overall incidence of ocular and nonocular adverse events and serious adverse events, including the Anti-Platelet Trialists' Collaboration-defined arterial thromboembolic events, was similar across treatment groups in the total patient population.

Conclusion: These findings suggest that the improvements achieved with IVT-AFL 2q4 and 2q8 over laser in vision and DRSS were robust and similar in patients with variable systemic disease control at baseline (HbA1c levels $\leq 8\%$ and $> 8\%$). Furthermore, the DRSS improvement indicates an effect of IVT-AFL not only on DME, but also on the underlying diabetic retinopathy. *Clinical Trial Registration Number:* NCT01331681, NCT01363440

OP 10 Entero-pancreatic endocrinology

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Severely disrupted islet function in mice with global deletion of GLP-1 and GIP receptors

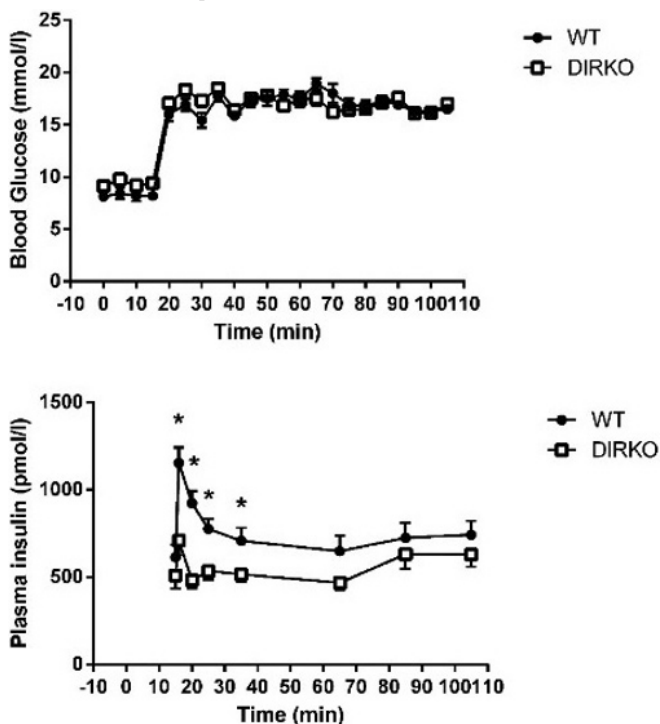
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Background and aims: The incretin hormones glucagon like peptide 1 (GLP-1) and glucose dependent insulintropic polypeptide (GIP) potentiate glucose stimulated insulin secretion. Both incretin hormones promote proliferation of pancreatic beta cells and prevent beta cell apoptosis. Incretins have also long been known to mediate the majority of insulin secretion during a meal or oral glucose challenge, however a role for the endogenous and basal incretin hormones in the long term regulation of beta cell function has not been characterized. We have therefore evaluated the role of basal incretin hormones on islet function by using mice with targeted disruption of both incretin hormone receptors.

Materials and methods: Double incretin receptor knockout (DIRKO) mice with global deletion of both the GLP-1 receptor and GIP receptor genes were used to study the effect of chronic deficiency of incretin action on beta cell function ex vivo and in vivo. Ex vivo insulin secretion was determined in static batch incubations of isolated islets from wild type and DIRKO mice with stimulatory glucose concentrations (16 mmol/l). In vivo insulin secretion was assessed by hyperglycaemic clamp in which, after a 20 minute baseline, mice were given a bolus injection of glucose (0.35mg/kg) together with infusion of 30% glucose in saline and clamped at a target blood glucose concentration of 16.7 mmol/l. The steady state period was between 20 and 105 minutes. Plasma insulin was measured at multiple time points throughout the steady state period. Insulin sensitivity from the clamp was determined by the insulin sensitivity index, calculated as the mean glucose infusion rate during the last 40 minutes of the steady state period divided by the mean plasma insulin concentration for the same period.

Results: Insulin secretion was markedly lower in islets from DIRKO mice compared to islets from wild type mice during static incubation of isolated islets with stimulatory glucose (16.7 mmol/l) (395 ± 80 vs 1171 ± 131 pmol/islet/hour, $p = 0.02$). In order to specifically study beta cell function in vivo, hyperglycaemic clamp experiments were performed in which mice were clamped with a target blood glucose concentration of 16.7 mmol/l. This target was closely achieved in both wild-type and DIRKO mice (17.0 ± 0.3 vs 17.1 ± 0.2 mmol/l). Insulin secretion during the hyperglycaemic clamp was severely impaired in DIRKO mice compared to control mice. The acute insulin response (AIR) was blunted in DIRKO mice compared to controls (199 ± 88 vs 540 ± 108 pmol/l, $p = 0.005$). The total area under the insulin secretion curve (AUC) during the steady state period was significantly lower in DIRKO mice compared to wild-type mice (25.5 ± 1.8 vs 37.0 ± 3.6 nmol/l*min, $p = 0.008$). Insulin sensitivity, derived from clamp data, was not significantly different between wild-type and DIRKO mice (0.029 ± 0.004 vs 0.036 ± 0.005 mg glucose/kg/min/pmol insulin).

Conclusion: Normal islet function is heavily dependent on both incretin hormones, whereas insulin sensitivity is not altered by genetic deletion of incretin hormone receptors



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GIP contributes to the protection from hypoglycaemia of DPP-4 inhibition

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Background and aims: Intensive glucose lowering therapies in type 2 diabetes (T2D) increases the risk of hypoglycaemia, which is associated with acute unpleasant consequences and increased long-term risk for cardiovascular diseases. A major defence mechanism to combat hypoglycaemia is glucagon counter-regulation; however, this mechanism is commonly compromised in persons with T2D. Therefore, there is a need to develop glucose-lowering therapies which sustain the endogenous counter-regulations to hypoglycaemia. Inhibition of dipeptidyl peptidase-4 (DPP-4) prevents the regulatory inactivation of the incretin hormones and is a glucose-lowering therapy in T2D with low risk for hypoglycaemia. We have previously shown that DPP-4 inhibition protects from insulin-induced hypoglycaemia in mice. The aim of the present study is to examine the mechanisms behind this protection and particularly the contribution of the incretin hormone glucose-dependent insulinotropic polypeptide (GIP).

Materials and methods: Anesthetized C57BL/6 mice were infused with 15 mU/kg/min of insulin and variable rates of glucose to target a steady state blood glucose of 2.5 mmol/L between minute 60 and 90 of the hyperinsulinemic hypoglycaemic clamp. To explore GIP in the endogenous defence against hypoglycaemia we performed hypoglycaemic clamps in GIPR^{-/-} mice lacking the GIP receptor (n=5) or wild type (wt) counterparts (n=7). To further explore the effect of GIP, wt mice were infused with synthetic GIP (50 pmol/kg/min) during hypoglycaemic clamp (n=10). Glucose, insulin and glucagon levels were measured and glucose infusion rate (GIR) during the clamp was estimated. To explore the relationship of GIP expression to glucagon secretion in humans, correlation analysis was performed in islet gene expression and secretion data from isolated human islets from 69 individuals. Values are presented as Mean±S.E and statistical significance assessed using Student's *t*-test.

Results: GIPR^{-/-} mice required increased GIR compared to matched wt animals to maintain blood glucose during the clamp (6.0±1.2 vs. 2.5±0.5 mg/kg/min; p=0.03). Both wt and GIPR^{-/-} mice had similar increases in endpoint glucagon after 90 minutes of clamp compared to baseline levels (2.3±0.5-

fold and 1.7±0.2-fold respectively; p=0.50). During GIP infusion, GIR was reduced to 4.2±0.9 compared to 6.9±0.8 mg/kg/min (p=0.040) in animals infused with saline. This was accompanied by a tendency towards an increase in glucagon levels at 60 (11.5±2.0 vs 6.2±1.9 pg/mL; p=0.072) and 90 (17.5±4.0 vs 8.6±2.1 pg/mL; p=0.062) minutes of the clamp. Clamp insulin levels did not differ between groups. In human islets, there was a significant positive correlation between GIP expression and glucagon secretion at 1 mM of glucose (rho=0.345, p=0.004).

Conclusion: GIPR^{-/-} mice were more sensitive to insulin-induced hypoglycaemia while GIP infusion during clamp protects wt mice from hypoglycaemia. Results from glucagon measurements suggests that GIP infusion protects from hypoglycaemia via increases in glucagon levels but also raise the possibility of glucagon independent mechanisms further explaining the protective mechanism of DPP-4 inhibition. Furthermore, the positive GIP expression correlation with increased glucagon secretion in human islets suggests relevance in humans. Based on these findings we conclude that GIP is a protective factor from hypoglycaemia and suggest that it contributes to the low risk of hypoglycaemia that can be seen with diabetes treatment using DPP-4 inhibition, thus elevating GIP levels.

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Altered enteroendocrine expression of glucagon and somatostatin in the gut of patients with type 2 diabetes compared with healthy matched controls

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Background and aims: Hormones secreted from enteroendocrine cells in the gut play a crucial role in regulation of whole-body glucose metabolism. Little is known about the distribution of these enteroendocrine cells along the intestinal tract, and it is currently unknown whether the distribution and/or phenotype of these cells is altered in type 2 diabetes (T2D). We aimed to evaluate the expression of products from enteroendocrine D, K and L cells, respectively, along the entire length of the small and large intestines in patients with T2D and in healthy control subjects

Materials and methods: We used double-balloon enteroscopy (DBE) to collect mucosal gut biopsies. The study involved 12 subjects diagnosed with T2D and 12 age and BMI-matched non-diabetic controls. All subjects underwent antegrade and retrograde DBE. Biopsies were collected from every 30 cm from pylorus to the ileocecal valve and from 6 specific sites in colon. RNA was isolated and analysed for expression of glucagon (GCG), peptide YY (PYY), glucose-dependent insulinotropic polypeptide (GIP) and somatostatin (SST). **Results:** Expression of markers for L cells (GCG, PYY) and K cells (GIP) showed significantly higher expression of L cell genes in the distal versus the proximal parts of the small intestine and significantly higher expression of the K cell marker (GIP) in the proximal part of the small intestine. Expression of the L cell marker (GCG) was significantly higher in the proximal small intestine in subjects with T2D compared to healthy controls (337% (113%;1002%) p<0.05). Expression of the D cell marker (SST) was significantly lower in the distal part of the small intestine in T2D subjects compared with healthy controls (21% (5%;27%) p<0.05).

Conclusion: Using DBE, we managed to collect biopsies along the entire length of the small and large intestines in patients with T2D and non-diabetic controls. Our results confirm the notion of higher expression of the L cell marker (GCG) distally, and higher expression of the K cell product (GIP) proximally in the small intestine. Importantly, we also show that intestinal expression of GCG and SST is altered in patients with T2D, a finding that might prove relevant in future prevention and treatment of the disease.

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Role of the somatostatin in the control of glucagon secretion by glucose and K_{ATP} channel blockers

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Background and aims: It is well established that glucose (G) inhibits glucagon release by pancreatic α -cells. However, it has sometimes been reported that high concentrations of the sugar (>20 mM) stimulate glucagon secretion. The mechanisms behind these effects are unknown. In particular, it is still unclear whether G controls glucagon release by modulating K_{ATP} channels of α - and non- α -cells within the islet and whether somatostatin (SST) released from δ -cells is involved in the control of glucagon release. In the present study, we evaluated the role of SST in the control of glucagon secretion by various G concentrations and K_{ATP} channel blockers.

Materials and methods: The effects of G and K_{ATP} channel modulators were tested on glucagon secretion of control islets and islets devoid of SST paracrine influence (islets pre-treated for 24 h with 200 ng/ml pertussis toxin (PTX) or from $Sst^{-/-}$ mice). Unless indicated, all the experiments were performed in the presence of a 6 mM mixture of amino acids (Mix AA: 2 mM alanine, 2 mM glutamine and 2 mM arginine).

Results: The role of SST in the control of glucagon secretion by various G concentrations (0, 1, 2, 5, 7, 15 and 30 mM) was first evaluated in incubation experiments with control islets or islets preincubated with PTX to remove the paracrine influence of SST. In control islets, G dose-dependently inhibited glucagon secretion with a maximal effect obtained already at around 7 mM. In PTX-pre-treated islets, glucagon secretion was larger at all G concentrations and the dose-response relationship for G-regulated glucagon secretion displayed a U-shape reflecting an inhibition of glucagon release at G concentrations up to 7 mM followed by a progressive decrease of the glucagonostatic effect at G concentrations >7 mM. Perfusion experiments with islets of $Sst^{+/+}$ or $Sst^{-/-}$ mice confirmed these results. Thus increasing the G concentration of the medium from 1 to 7 or 30 mM equally inhibited glucagon release of $Sst^{+/+}$ islets, whereas G7 inhibited glucagon release more potently than G30 in $Sst^{-/-}$ islets. Moreover, increasing the G concentration of the perfusion medium from 7 to 30 mM did not affect glucagon release of $Sst^{+/+}$ islets, whereas it stimulated that of $Sst^{-/-}$ islets. These results suggest that SST is partially involved in the glucagonostatic effect of high G concentrations. We next studied the role of SST in the control of glucagon secretion by tolbutamide (Tolb, a K_{ATP} channel blocker). In $Sst^{+/+}$ islet, Tolb (500 μ M) did not affect glucagon release at G1 and slightly stimulated it at G7. By contrast, in $Sst^{-/-}$ islets, Tolb stimulated glucagon secretion both at G1 and G7. The glucagonotropic effect of Tolb in $Sst^{-/-}$ islets was much more evident in the absence of Mix AA. It is unrelated to Epac2 activation because a much lower concentration of Tolb (10 μ M) and gliclazide (10 μ M) which do not activate Epac2 also strongly stimulated glucagon release of $Sst^{-/-}$ islets perfused with G7 and without MixAA.

Conclusion: G dose-dependently inhibits glucagon release of control islets. This inhibition is independent of SST for intermediate G concentrations (0–7 mM) but starts to involve SST for G concentrations >7 mM. Pharmacological closure of K_{ATP} channels controls glucagon secretion by two mechanisms: a direct stimulation of α -cells (independent of Epac2 activation and observed in the absence of SST influence) and an indirect inhibition via SST released from δ -cells.

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Effect of glucose and free fatty acid on PC1/3-PC2 expression in pancreatic alpha cell

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Background and aims: An intra-islet incretin system has been recently suggested to operate through modulation of the expression and activity of proconvertase 1/3 and 2 (PC1/3, PC2) in pancreatic alpha-cell accounting for local release of GLP-1. Little is known, whether this alpha-cell activity can be affected by the metabolic alterations occurring in type 2 diabetes, such as hyperglycaemia, hyperlipidemia or hyperglucagonemia.

Materials and methods: AlphaTC1/6 cells from a mice pancreatic cell line were incubated in the presence of two glucose (G) concentration (5.5 and 16.7 mM) for 16 h with or without free fatty acid (FA, 2:1 palmitate:oleate,

0.5 mM), IL6 (200 ng/ml) or glucagon (GLU: 250 pg/ml). GLP-1 secretion was measured by ELISA and expression of PC1/3 and PC2 by RT-PCR and western blot; cell viability was determined by MTT method, Reactive Oxygen Species generation (ROS) by H2DCFDA fluorescence and apoptosis by Annexin staining and Propidium Iodine (PI) fluorescence.

Results: Upon 16.7G incubation, GLP-1 secretion (total and active) was significantly increased ($130 \pm 9\%$ and $158 \pm 19\%$, respectively, $p < 0.04$, all vs 5.5G) in parallel with a significant increment in PC1/3 expression (RNA: 1.37 ± 0.06 folds and protein: $138 \pm 10\%$, both $p < 0.02$), a slight increase in cell viability ($116 \pm 3\%$, $p < 0.001$) and ROS generation ($118 \pm 5\%$, $p < 0.001$) and by a decrement in PC2 expression (RNA: 0.51 ± 0.06 , $p < 0.05$) with no change in cell apoptosis ($105 \pm 4\%$). No change was observed upon incubation with mannitol. When cells were incubated at 5.5G + FA, also an increment in GLP-1 secretion ($173 \pm 21\%$, $p < 0.02$) and PC1/3 expression was observed (RNA: 2.07 ± 0.24 folds, $p < 0.006$) together an increment in ROS generation ($132 \pm 8\%$, $p < 0.002$), a decrement in cell viability ($47 \pm 2\%$, $p < 0.001$), and a modest increment in apoptosis (Annexin: $115 \pm 5\%$, $p < 0.03$; PI: $109 \pm 5\%$). When incubated with 16.7G + FA, the increment in GLP-1 secretion was reduced to basal ($107 \pm 6\%$ vs. 5.5G), accompanied by an increment in apoptosis (Annexin: $152 \pm 11\%$, $p < 0.001$; PI: $142 \pm 9\%$, $p < 0.001$) and ROS generation ($139 \pm 9\%$, $p < 0.001$). This was also observed with IL6 (GLP-1 secretion: 5.5G + IL6: $156 \pm 12\%$ vs. 16.7G + IL6: $111 \pm 25\%$; PC1/3: 5.5G + IL6: 2.78 ± 1.04 vs 16.7G + IL6: 6.56 ± 1.37), but in this case, no modification in ROS generation or apoptosis was observed when compared to 16.7G. The presence of GLU did not modify any of the parameters studied.

Conclusion: These data suggest that under hyperglycaemic, hyperlipidaemic or inflammatory conditions, alpha cells can increase expression PC1/3 and activate GLP-1 secretion, which may contribute protecting both alpha and beta-cells from glucose and lipotoxicity, while this effect seems to be lost in the presence of both pathophysiological conditions.

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Gp130 receptor signalling mediates alpha cell dysfunction in a rodent model of type 2 diabetes

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Background and aims: Dysregulated glucagon secretion accompanies islet inflammation in type 2 diabetes. We recently discovered that IL-6 stimulates glucagon secretion from human and rodent islets. IL-6 family cytokines require the gp130 receptor to signal. In this study we elucidated the effects of a cell gp130 receptor signaling on glycaemic control in type 2 diabetes.

Materials and methods: IL6 family cytokines were analyzed in islets in two rodent models of T2D. Gp130 receptor signalling was assessed in primary α cells. Furthermore, a cell specific gp130 knock out (agp130KO) mice were generated and subjected to streptozotocin (STZ) alone, high fat diet (HFD) alone, and STZ plus HFD to study how a cell dysfunction contributes to glycaemic control. Glucose tolerance tests, insulin tolerance tests, and hyperinsulinemic-euglycemic clamps were performed to assess a cell function, β cell function, and insulin sensitivity.

Results: IL-6 family cytokines were elevated in islets in rodent models of this disease. Gp130 receptor activation induced STAT3 phosphorylation in primary α cells and stimulated glucagon secretion. agp130KO mice showed no differences in glycaemic control, a cell function or a cell mass. However, when subjected to STZ alone agp130KO mice showed improved glucose tolerance. Further, when subjected to STZ plus HFD to induce islet inflammation and pathophysiology modelling type 2 diabetes, agp130KO mice had reduced fasting glycaemia, improved glucose tolerance, reduced fasting insulin, and improved a cell function despite decreased fasting GLP-1 levels. Hyperinsulinemic-euglycemic clamps revealed no differences in insulin sensitivity. **Conclusion:** We conclude that during T2D, activation of a cell gp130 receptor signaling causes impaired α cell function, promoting hyperglycaemia. Despite decreased systemic GLP-1 levels in agp130KO mice (likely due to receptor deletion in L cells), activation of a cell gp130 receptor signalling in a setting of reduced β cell mass and HFD-induced insulin resistance had detrimental effects on normal α cell function and glycaemic control. Antagonism of a cell gp130 receptor signaling may be useful for the treatment of T2D.

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Trajectories of glycaemic traits in south Asians and whites before diabetes diagnosis: a longitudinal analysis from the Whitehall II study

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Background and aims: An increased risk of type 2 diabetes among south Asians compared to whites is well established. Most of the literature suggests that this is related to lower insulin sensitivity among south Asians however the role of insulin secretion is rarely assessed. Therefore, we aimed to investigate ethnic differences in trajectories of fasting (FPG) and 2-h post-load plasma glucose (2hPG), log-transformed homeostasis model assessment insulin sensitivity (HOMA-S) and secretion (HOMA-B) prior to the diagnosis of type 2 diabetes.

Materials and methods: We analysed trajectories of glycaemic traits before the diagnosis of diabetes by fitting mixed-effects models to longitudinal data (with up to 4 repeat measures within individuals) from 101 south Asian and 764 white participants of the Whitehall II study, who developed type 2 diabetes during follow-up between 1992 and 2009.

Results: South Asians had an almost 4 times increased risk of incident diabetes compared to whites during follow-up (26.4% vs 10.2%, $P < 0.001$). According to age and sex-adjusted mixed-effects models, South Asians had a faster increasing FPG trajectory before diagnosis (slope difference: 0.34 mmol/l per decade, 95% CI: 0.05, 0.64; $P = 0.022$) and 0.36 mmol/l (95% CI: 0.10, 0.63; $P = 0.007$) higher FPG levels at diagnosis than whites. There were no differences in 2hPG trajectories between the two ethnic groups. South Asians had significantly lower HOMA-S already 15 years before diagnosis and the difference increased even further until diagnosis (difference at diagnosis: 0.30, 95% CI: 0.14, 0.46; $P < 0.001$). HOMA-B trajectories had different quadratic characteristics ($P = 0.04$ for ethnicity \times time² interaction). It increased in both ethnicities until 7 years before diagnosis however the increase was faster in whites, while it followed a similar decreasing trajectory thereafter until diagnosis. Differences in FPG and HOMA-B trajectories were robust to adjustment for measures of obesity and lifestyle measures.

Conclusion: Insulin sensitivity falls more rapidly among south Asians than whites. In contrast to whites, who could increase insulin secretion up to 7 years before diagnosis, this compensatory mechanism is can be hardly seen in south Asians, hence a more rapidly increasing FPG trajectory develops prior to the diagnosis of diabetes. These findings extend our prior observation of inadequate β -cell compensation with aging in healthy south Asians.

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Food sources of fat may clarify the earlier inconsistent role of dietary fat intake for incidence of type 2 diabetes

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Background and aims: Dietary fats could affect glucose metabolism and insulin sensitivity and may thereby have a crucial role in the development of type 2 diabetes (T2D). Studies have indicated that replacing saturated fat with monounsaturated and polyunsaturated fats might be favorable in the prevention of T2D. In line with this, plant sources of fat have been suggested to be a better choice compared with animal sources. Indeed, high intakes of red meat and meat products show positive associations with risk of T2D. Nevertheless, several epidemiological studies have indicated that a high intake of dairy products may be protective. Subsequently, the importance of dietary fat content and food sources of fat remains to be clarified. Our aim was to exam-

ine intakes of main dietary fat sources, classified according to fat content, in relation to incident T2D.

Materials and methods: In total 26 930 individuals (60% women), 45–74 years, from the population-based Malmö Diet and Cancer cohort, were included. Dietary data was collected with a modified diet history method. During 14 years follow-up, 2860 incident T2D cases were identified. Cox proportional hazards regression model was used to estimate hazard ratios (HR) of diabetes incidence in quintiles of energy adjusted dietary intakes. The multivariate model included adjustments for age, sex, season, diet assessment method version, total energy intake, BMI, leisure time physical activity, smoking, alcohol consumption and education.

Results: High intake of high-fat dairy products was associated with lower incidence of T2D (HR for the highest (median=8 portions/day) compared with the lowest (median=1 portion/day) intake quintile: 0.77; 95% CI: 0.68–0.87; P for trend < 0.001). Concerning intakes of specific high-fat dairy foods, cream and high-fat fermented milk were inversely associated with risk of T2D ($P < 0.01$). High intake of low-fat dairy products was associated with increased risk (P for trend=0.01), but this association disappeared after additional adjustment for protein intake (P for trend=0.37). High intakes of meat and meat products were, regardless of fat content associated with increased risk (HR: 1.09; CI: 0.97–1.24; P for trend=0.04 and HR: 1.25; 95% CI: 1.11–1.41; P for trend < 0.001 , for high- and low-fat meat respectively)(median intakes in the highest quintiles=90, 80 g/day).

Conclusion: Our observations may contribute to clarifying previous findings regarding dietary fats and their food sources in relation to T2D. The decreased risk at high intakes of high-fat dairy products, but not of low-fat dairy products, indicate that dairy fat, at least partly, explains observed protective associations between dairy intake and T2D. Meat intake was associated with increased risk independently of fat content.

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Movement during sedentary time is associated with metabolic outcomes

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Background and aims: Studies have shown that more sedentary time is associated with adverse health effects including metabolic syndrome, type 2 diabetes, cardiovascular diseases, and increased mortality risk. Next to total amount of sedentary time other aspects of sedentary behaviour, such as the pattern in which sedentary time is accumulated, have been associated with health outcomes. During sedentary time the degree of movement of the upper body can differ between individuals and this could be another relevant aspect of sedentary behaviour. Whether movement during sedentary time is meaningful for health outcomes is not clear and therefore this study aims to examine the association between movement during sedentary time and metabolic outcomes.

Materials and methods: 631 participants (aged 73–98 years) of the AGESII-Reykjavik Study wore a triaxial accelerometer (ActiGraph GT3X) for 7 consecutive days. Movement during sedentary time was defined as any activity in the anteroposterior and/or mediolateral axes with an intensity ≥ 100 counts per minute (cpm), during sedentary time (< 100 cpm in the vertical axis). Metabolic outcomes included body mass index (BMI), waist circumference (WC), levels of HDL-cholesterol (HDL), triglycerides (TG), fasting glucose (FG) and C-reactive protein (CRP).

Results: Linear regression analysis showed that compared with those who had the most minutes with movements during sedentary time, participants with fewer movement minutes (quartiles 3, 2, 1) had a higher BMI ($B = 1.43$; $B = 1.97$; $B = 3.38$; all $p < 0.05$) and an up to 8.3 cm larger WC ($B = 4.66$; $B = 5.25$; $B = 8.30$; all $p < 0.05$) after adjusting for demographic and health factors, sedentary time, and MVPA. Fewer movement minutes were also associated with lower levels of HDL (Q3 $B = -0.11$; Q1 $B = -0.13$; all $p < 0.05$) and higher TG levels ($B = 1.10$; $B = 1.09$; $B = 1.10$; all $p < 0.05$, back transformed from log scale), but not with FG and CRP.

Conclusion: Movement during sedentary time was associated with metabolic outcomes; an up to 3.4 kg/m² higher BMI and 8.3 cm larger WC was seen in participants with less movement minutes. These findings suggest that movement during sedentary time could be a relevant aspect of sedentary behaviour.

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Association of restrictive ventilatory dysfunction with development of prediabetes and type 2 diabetes in Koreans

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Background and aims: Accumulating evidence have suggested that reduced lung function, especially restrictive pattern, is associated with insulin resistance and type 2 diabetes. However, the causal direction between reduced lung function and type 2 diabetes remains unclear and there is a paucity of longitudinal studies in Asian populations. We investigated whether restrictive ventilatory dysfunction is a predictor for development of prediabetes and type 2 diabetes in Koreans.

Materials and methods: We analyzed the clinical and laboratory data of 17,144 Korean adults (age 20–79 years, 37.8% women) who underwent routine medical check-ups in 2008 (baseline) and again in 2012–13 (follow up) with a median 4.9-year (range 3.0–5.9 years) interval. Patients who had diabetes at baseline were excluded. Results of spirometry were categorized into three groups; normal (FEV1/FVC ≥ 0.70 , FVC $\geq 80\%$ predicted), obstructive ventilatory dysfunction (OVD; FEV1/FVC < 0.70), and restrictive ventilatory dysfunction (RVD; FVC $< 80\%$ predicted, FEV1/FVC ≥ 0.70). At baseline, 1518 subjects (9.4%) had RVD and 770 (4.8%) had OVD. Prediabetes was defined as fasting plasma glucose 5.6–6.9 mmol/l or HbA1c 39–46 mmol/mol [5.7–6.4%].

Results: Subjects with RVD had higher age, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, and fasting glucose level at baseline compared to those with normal ventilatory function. Among the 16,195 participants who did not have diabetes at baseline, a total of 640 subjects (4.0%) developed type 2 diabetes during the follow-up period. Compared to subjects with normal ventilatory function, the subjects with RVD had a higher incidence of type 2 diabetes (3.7% vs. 6.3%, $P < 0.001$) but subjects with OVD did not (3.7% vs. 4.8%, $P = 0.119$). On multiple logistic regression analysis, the OR of type 2 diabetes in subjects with RVD was significantly increased (1.47 [1.16–1.87], $P = 0.001$) after adjusting for age, sex, exercise, drinking, smoking, and blood pressure. However, further adjustment for BMI, waist circumference, and baseline glucose level attenuated the OR to become insignificant (1.17 [0.90–1.52], $P = 0.251$). Since subjects with RVD already had higher prevalence of prediabetes at baseline, we confined the analysis to subjects with normal fasting glucose and HbA1c levels at baseline. Among those 9461 participants, a total of 2288 subjects (24.2%) developed prediabetes and 37 (0.4%) developed type 2 diabetes during the follow-up. The OR of progression to prediabetes or diabetes in subjects with RVD was 1.30 (1.12–1.51, $P = 0.001$) after controlling age, sex, exercise, drinking, smoking, and blood pressure. The OR remained significantly increased after further adjustment for BMI, waist circumference, and baseline glucose level (1.26 [1.07–1.47], $P = 0.004$).

Conclusion: Our results indicate that restrictive, but not obstructive, ventilatory dysfunction is independently associated with development of prediabetes and may precede the development of type 2 diabetes.

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Lifestyle factors and genetic predisposition to obesity in people from Pakistan: the PROMIS study

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Background and aims: Most complex trait genetics studies have focused on populations of European ancestry. However, conducting such studies in South Asians is important because their lifestyles are becoming increasingly westernized, their genetic architecture is somewhat distinct from Europeans, and the burden of complex diseases is growing rapidly. In 14,786 adults (N=7,354 with myocardial infarction (MI)) from the Pakistan Risk of Myocardial Infarction Study (PROMIS) we tested if: a) 31 loci, established in Europeans to predispose to higher BMI, also do so in Pakistanis, and b) physical activity, smoking and cooking oil type modify the effects of these loci.

Materials and methods: A genetic risk score (GRS) comprised of 31 BMI-susceptibility loci was calculated by summing the BMI-associated alleles at each variant. Physical activity, smoking, and cooking oil type were assessed using self-administered questionnaires. Generalized linear models and logistic regression were used to examine associations and interactions (SNP*lifestyle factor), with BMI as the outcome. Analyses were adjusted for age, age², sex, MI (yes/no), and population substructure.

Results: In the total sample, each additional GRS risk allele was positively associated with BMI (0.07 (SE= 0.1) kg/m²/risk allele; $P < 0.0001$). In interaction analyses, physical activity modified the effect of the GRS on BMI in the total sample ($P_{\text{interaction}} = 0.009$); the BMI-raising allele was associated with 0.11 (SE= 0.02) kg/m² higher BMI in inactivity participants ($P < 0.0001$), whereas the GRS effect in moderately and in very physically active participants was 0.07 (SE= 0.01; $P < 0.0001$), and 0.02 (SE= 0.03; $P = 0.59$) kg/m² per allele respectively. Although the interaction effect estimates were essentially the same in cases and controls as in the full sample, the tests of interaction were not statistically significant by case/control strata ($P_{\text{interaction}} = 0.07$ and 0.08 respectively), possibly because of the smaller sample sizes (Fig. 1). In individual SNP analyses, the rs2815752 NEGR1 variant modified the association of physical activity with BMI in the total sample ($P_{\text{interaction}} = 0.002$), and in controls ($P_{\text{interaction}} = 0.035$) and cases ($P_{\text{interaction}} = 0.035$) separately.

Conclusion: Physical activity may modify the genetic predisposition to obesity in Pakistanis, the latter observation being consistent with earlier reports in European ancestry populations.

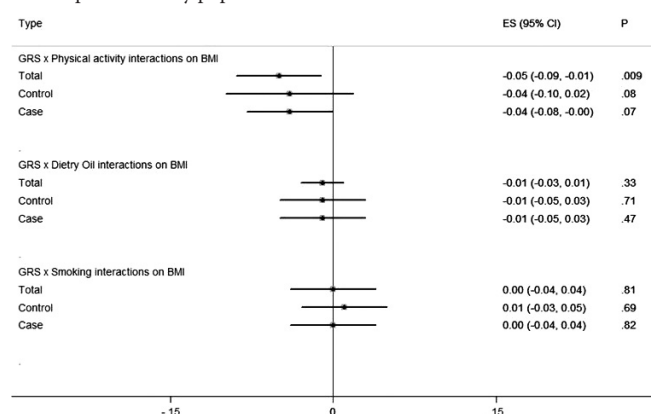


Figure 1 Forest plot shows the interaction of GRS x exposure (physical activity, dietary oil type and smoking) on BMI in total sample, control and cases in PROMIS study.

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Effect of lifestyle modification on the prevention of type 2 diabetes and impaired glucose tolerance in a young healthy urban South Asian populationJ. Karalliedde¹, M. Wijesuriya², L. Vasantharajah², M. Gulliford³, G. Viberti¹, L. Gnudi²;¹Diabetes, King's College London, UK, ²Diabetes, Diabetes Association of Sri Lanka, Colombo, Sri Lanka, ³King's College London, UK.

Background and aims: There is an increasing incidence of type 2 diabetes mellitus (T2DM) and related cardiovascular disease (CVD) in young South Asian subjects. Several studies have confirmed that lifestyle modification (LSM) is very effective in preventing T2DM in older subjects with impaired glucose tolerance (IGT). The effect of LSM on the prevention of cardio-metabolic disease in young healthy urban South Asian subjects is unknown.

Materials and methods: A randomised controlled clinical trial to compare an intensive 3-monthly LSM (I-LSM) with a less-intensive 12 monthly LSM (LI-LSM) for a primary composite cardio-metabolic endpoint of new onset T2DM, IGT, impaired fasting glycaemia, hypertension, initiation of lipid lowering therapy and cardio-renal disease in subjects aged 5 to 40 years with at least 2 of the following risk factors: raised body mass index, raised waist circumference (WC), first degree family history of T2DM and physical inactivity. A cluster sampling strategy was used to select a representative sample of healthy at risk subjects with two or more of the above risk factors. We randomised 4606 subjects of whom 3,685 (48% males) qualified for analyses. The study was performed at a single centre in Colombo, Sri Lanka between 2010 to 2013. Intervention: Each subject received LSM advice aimed at reducing weight, improving diet and increasing physical activity 3 monthly (I-LSM) or 12 monthly (LI-LSM) for 4 years.

Results: There were no significant baseline differences in anthropometric, clinical and demographic measures between groups [I-LSM (n=1807, mean \pm SD age, 22.4 \pm 10 yrs.) and LI-LSM (n=1878 age, 22.4 \pm 9.8 yrs)]. The cumulative incidence of the primary end point after 4 years was n=270 in I-LSM vs. n=302 in LI-LSM, a 9% (95% CI 1.0% to 16%) relative risk reduction (RRR), which was independent of baseline age, gender, p=0.02. Similarly there was a significant 26% RRR (95% CI 2% to 44%) in new onset T2DM p=0.04, and 18% RRR (95% CI 7% to 28%) in new onset IGT p=0.002. I-LSM did not significantly reduce the other components of the primary composite endpoint.

Conclusion: Our results demonstrate for the first time that in a young healthy but at risk South Asian population intensive LSM significantly reduces the development of T2DM, IGT and a composite endpoint of cardio-metabolic disease. These results highlight the importance of intervening early with lifestyle modification to prevent and reduce the burden of T2DM in young at risk subjects.

Clinical Trial Registration Number: SLCTR/2008/World Health Organization (WHO)

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OP 12 The many faces of advanced glycation

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Exogenous and endogenous hydrogen sulfide protects the cardiomyocyte cell line H9c2 from methylglyoxal-mediated damageT. Ghela¹, J.G. Mabley²;¹Brighton and Sussex Medical School,²School of Pharmacy & Biomolecular Sciences, University of Brighton, UK.

Background and aims: Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound, has been implicated as a mediator of diabetic cardiovascular complications. MGO has already been shown to cause endothelial cell dysfunction as well as cardiomyocyte contractile dysfunction via increased cellular oxidative stress. Hydrogen sulfide is a gaseous transmitter found in the vasculature and synthesised from the amino acid L-cysteine. Hydrogen sulfide has been demonstrated to have a protective role in the vasculature acting as a vasodilator and protecting cells from increased oxidative stress. Hydrogen sulfide has already been shown to protect endothelial cells from MGO-mediated damage. The aim of this study is to investigate whether hydrogen sulfide generated exogenously by a chemical donor or endogenously from L-cysteine is also able to protect cardiomyocytes from MGO-mediated dysfunction.

Materials and methods: The cardiomyocyte cell line H9c2 was exposed to increasing concentrations of MGO (0.1–1 mM) for 24h prior to measuring cell viability and apoptosis. In a second series of experiments H9c2 cells were exposed to MGO 0.6 and 0.8 mM in combination with 0.25 or 0.5 mM sodium hydrogen sulfide or L-cysteine. Cell viability was determined using the MTT assay and cell death by propidium iodide/Hoechst staining. Endoplasmic Reticulum (ER) stress by Western blotting for CHOP.

Results: Methylglyoxal dose-dependently reduced H9c2 cell viability. MGO 0.6 and 0.8 mM reduced cell viability to 55 \pm 3% and 47 \pm 2% respectively, sodium hydrogen sulfide protected against this loss of cell viability with 0.25 mM returning viability to 65 \pm 5% and 57 \pm 4% and 0.5 mM returning viability to 70 \pm 4% and 63 \pm 3% respectively (p<0.05 vs. MGO alone). Similarly L-cysteine also protected against the MGO-mediated loss of cell viability with 0.25 mM returning viability to 79 \pm 5% and 61 \pm 3% and 0.5 mM returning viability to 106 \pm 5% and 92 \pm 3% respectively (p<0.05 vs MGO alone). To determine if this protection by L-cysteine was mediated by endogenous synthesis of hydrogen sulfide we used an inhibitor of the synthesizing enzyme cystathionine-gamma-lyase, DL-propargylglycine (PAG) 10 mM, pretreating the cells for 2h prior to treatment with MGO and L-cysteine. In the absence of PAG 0.25 and 0.5 mM L-cysteine protected against MGO 0.8 mM; increasing viability by 9 \pm 2% and 49 \pm 2% respectively but following PAG pretreatment this protection was reduced to -0.7 \pm 0.4% and 27 \pm 2% (p<0.05). MGO was found to increase apoptosis levels in H9c2 cells by a mechanism independent of ER stress. MGO also increases H9c2 cell necrosis levels. Again both sodium hydrogen sulfide and L-cysteine protected against this MGO-mediated increase in apoptosis and necrosis.

Conclusion: Hydrogen sulfide generated either directly from a chemical donor or indirectly from the endogenous substrate L-cysteine provided significant protection against MGO-mediated decrease in H9c2 cell viability and increased necrosis and apoptosis. This data suggests that not only will hydrogen sulfide protect endothelial cells but will also directly protect cardiomyocytes from methylglyoxal-mediated damage. These data suggest that hydrogen sulfide donors or therapies designed to increase endogenous production of hydrogen sulfide may be useful in reducing diabetic cardiovascular complications.

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Metabolic modelling of methylglyoxal formation, metabolism and AGE formation in vascular endothelial cells in model hyperglycaemia in vitro

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Background and aims: Methylglyoxal (MG) is a reactive dicarbonyl, glucose-derived metabolite and precursor of quantitatively major advanced glycation endproducts (AGEs). Increased MG-derived AGEs in vascular endothelial cells likely contributes to endothelial cell dysfunction and the development

of vascular complications of diabetes. Glyoxalase1 (Glo1) and glyoxalase 2 (Glo2) of the cytoplasmic glyoxalase system metabolises MG and prevents AGE formation. The aim of this investigation was to produce a mathematical model of MG concentration and related AGE formation in human vascular endothelial cells in vitro from experimental measurements of glucose metabolism and activities of Glo1 and Glo2 in normal glucose concentration and also high glucose concentration to model hyperglycaemia. The model is then validated by experimental measurement of cellular MG and AGEs. Exposure to increased AGEs may thereby be predicted for a given level of hyperglycaemia and the level of pharmacological induction of Glo1 induction required to prevent increased AGEs predicted.

Materials and methods: Human microvascular endothelial HMEC-1 cells were incubated in MCDB131 media with 10% serum containing low glucose or high Glucose (5 mM, LG and 30 mM, HG, respectively) for 6 days at 37°C. Similar experiments were performed with human aortic endothelial cells (HAECs) in primary culture with 5 mM or 20 mM glucose. Rate of glucose consumption, activities of Glo1 and glyoxalase 2, cellular and medium concentrations of MG, flux of MG (measured as the surrogate accumulation of D-lactate), D-lactate metabolism and cell protein content and flux of the major MG-derived AGE, hydroimidazolone MG-H1 were determined. Single and two compartment models of MG metabolism by the glyoxalase system and AGE formation were constructed using the COPASI programme.

Results: Incubation of HMEC-1 cells in HG produced a progressive decrease in Glo1 activity of 31% after 6 days. The rate of glucose metabolism was increased 70% and the flux of formation of MG was increased 66%. A mathematical model taking into account flux of MG formation, reversible binding of MG to GSH and protein thiols, metabolism by Glo1 and rate of MG glycation predicted an increase in cellular MG concentration and related AGEs in HG of 134%. Experimental validation produced similar results: increased in MG content of cells (pmol/106 cells) of 114% (LG, 2.08 ± 1.15 ; HG, 4.76 ± 0.38 , $P < 0.02$) and increased flux of formation of the major MG-derived AGE, MG-H1, of 102% in HG ($P < 0.05$). Similar effects were found for HAECs. This can be corrected by a 3-fold induction of Glo1 expression which is achieved by small molecule Glo1 inducers.

Conclusion: MG-derived AGE formation in human vascular endothelial cells in vitro are increased in model hyperglycaemia by synergistic effects of increased formation of MG from increased anaerobic glycolysis and decreased metabolism of MG by decreased Glo1 activity. Correction of increased MG and AGE formation in hyperglycaemia is likely within the pharmacological capability of Glo1 inducers currently under investigation.

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Intracellular accumulation of methylglyoxal alters the collagen expression profile of L6 myoblasts

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Background and aims: We have previously shown that down-regulation of the Methylglyoxal (MG) detoxifying glyoxalase system by siRNA mediated knockdown of (GLO1) results in impaired endocytosis of the transmembrane GLUT4 transporter leading to a prolonged and exaggerated cellular glucose influx. The consequences of this finding were analysed with special focus on the expression of extracellular matrix components in rat L6 myoblasts stably overexpressing the GLUT4 transporter.

Materials and methods: Detection of proteins and mRNA expression levels were carried out by western blot and quantitative real time PCR (qRT-PCR) analysis following intracellular accumulation of MG by siRNA mediated knockdown of Glo1 under hyperglycaemic conditions. mRNA levels and protein levels were determined 72 hrs after knockdown of Glo1. Normo- and hyperglycaemic controls were prepared following the same incubation time.

Results: The fibrillous collagens-1, -3, and -5 were significantly up-regulated on the mRNA-level ($p < 0.0005$ vs. scrambled control), the increase in collagen-1 expression was also demonstrated on the protein level ($p < 0.05$ vs. scrambled control), whereas for collagen-5 the increased expression was not detected on the protein level. For collagen-4 an increased mRNA expression was already detected following hyperglycaemia ($p < 0.05$ vs. normoglycaemia), which was more pronounced following Glo1 knockdown ($p < 0.0005$ vs. scrambled control). Regarding regulators of extracellular matrix turnover MMP-9 was significantly up-regulated ($p < 0.001$ vs. scrambled control) on mRNA level, but not on protein level. A reduced expression of the cross-linking enzyme PLOD2 ($p < 0.0005$ vs. scrambled control) and a slightly but non-significantly increased LEPREL2 were detected on protein level. DPSYL2,

which is of influence in cytoskeletal remodeling, is up-regulated on mRNA and protein level ($p < 0.0005$ vs. scrambled control, $p < 0.05$ vs. hyperglycaemia, respectively).

Conclusion: The results of this experimental research further elucidate the effect of exaggerated glucose influx after MG induced prolonged GLUT4 presence in the cell membrane on the cytoskeleton and extracellular matrix. The impact of expression of fibrillous and basal lamina associated collagens may account for the observations commonly detected in myofibrosis.

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Synergistic hepatotoxic effects with a combination of methylglyoxal and palmitic acid

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Background and aims: Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound, has been implicated as a central mediator of diabetic complications. Recently diabetes has been found to be an independent risk factor for development of liver disease including non-alcoholic fatty liver disease (NAFLD). Hepatocyte exposure to MGO or the saturated fatty acid, palmitic acid (PA) results in a loss of cell viability, increase in apoptosis and increased inflammation as demonstrated by IL-8 release. The aim of this study was therefore to determine if a combination of methylglyoxal and palmitic acid can synergistically damage hepatocytes and increase inflammation.

Materials and methods: The human hepatocyte cell line, HepG2, was exposed to MGO (0.6 mM) in combination with PA (3 or 10 μ M) for 24h. Cell viability was determined using the MTT assay and cell death by propidium iodide/Hoechst staining. Inflammation was assessed by IL-8 release and Endoplasmic Reticulum (ER) stress by Western blotting for CHOP.

Results: Hepatocytes exposed to a combination of MGO and PA had a lower cell viability and increased levels of apoptosis as compared to exposure to either component alone. Exposure to MGO 0.6 in combination with PA 3 μ M decreased cell viability to $78 \pm 3\%$ as compared to $81 \pm 1\%$ and $98 \pm 2\%$ for MGO and PA alone ($p < 0.05$), and also increased apoptosis from $0.3 \pm 0.05\%$ to $2.3 \pm 0.2\%$ as compared to $0.7 \pm 0.1\%$ and $0.9 \pm 0.1\%$ for MGO and PA alone ($p < 0.05$). Exposure to MGO 0.6 in combination with PA 10 μ M decreased cell viability to $74 \pm 1\%$ as compared to $81 \pm 1\%$ and $97 \pm 2\%$ for MGO and PA alone ($p < 0.05$), and also increased apoptosis to $3.9 \pm 0.1\%$ as compared to $0.7 \pm 0.1\%$ and $1.5 \pm 0.5\%$ for MGO and PA alone ($p < 0.05$). The increase in apoptosis may be accounted for by increased ER stress in response to the combination of MGO and PA as compared to that seen with PA alone. However, no such synergy was seen with inflammation only an additive effect was observed with IL-8 release increased by 49 ± 13 and $46 \pm 8\%$ with the combination of MGO 0.6 mM and PA 3 or 10 μ M respectively. Whereas IL-8 increased by $30 \pm 5\%$ with MGO 0.6 mM and 13 ± 6 and $8 \pm 7\%$ for PA 3 and 10 μ M when applied alone.

Conclusion: The synergistic effects of methylglyoxal and with relatively low physiological concentrations of palmitic acid on hepatocyte viability and apoptosis as well as the additive effects on inflammation may increase the risk of hepatotoxic effects being observed in diabetics. This may also account for the increased risk diabetics have of developing liver diseases such as NAFLD and strategies to not only lower saturated fatty acid levels but also methylglyoxal levels may prove effective in preventing these complications.

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The effect of hypertriglyceridaemia and metformin on reactive dicarbonyls in heart and kidney in a model of metabolic syndrome

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Background and aims: Biogenesis of reactive dicarbonyls is related to glycaemic and lipid status in diabetes and higher plasma levels of methylglyoxal are observed in diabetic patients. Excessive generation of dicarbonyls leads to production of advanced glycation end products (AGE), activates inflammatory pathway, increases oxidative stress and can play a key role in the development of diabetic vascular complications. Dicarbonyls determination could be early biomarker risk for these complications. Metformin, the most widely prescribed glucose- and lipid-lowering agent for treatment of type 2 diabetes, has also been proposed as a scavenger of methylglyoxal. However its effect on

individual dicarbonyls metabolism in tissue is not fully clarified. In our study we analyzed level of individual reactive dicarbonyls - methylglyoxal (MG), glyoxal (GL) and 3-deoxyglucosone (3-DG) in relation to lipid disorders and after metformin administration in non-obese rats with hereditary hypertriglyceridemia (HHTg rats).

Materials and methods: Adult Wistar rats (controls) and HHTg rats, which exhibit genetic fixed hypertriglyceridemia, tissue insulin resistance, impaired glucose tolerance, hyperinsulinemia and ectopic lipid accumulation, were fed a standard laboratory diet with or without metformin (300mg/kg b.wt.) for 4 weeks. The concentration of dicarbonyls were determined by derivatisation with 1,2-diamino-4,5-dimethoxy-benzene and HPLC-method with fluorescence detection.

Results: Compared with controls, HHTg rats exhibited markedly elevated serum levels of triacylglycerol (4.05 ± 0.39 vs 1.88 ± 0.23 mmol/l, $p < 0.001$), FFA (0.97 ± 0.19 vs 0.47 ± 0.13 mmol/l, $p < 0.01$) and hepatic triglycerides accumulation (13.87 ± 2.23 vs 4.32 ± 0.70 μ mol/g, $p < 0.001$). In HHTg rats we observed markedly increased serum level of MG ($p < 0.01$). Concentrations of individual reactive dicarbonyls in myocardium (MG: 14.99 ± 0.78 vs 8.66 ± 1.28 nmol/mg, $p < 0.001$; GL: 3.53 ± 0.51 vs 1.22 ± 0.42 nmol/mg, $p < 0.01$; 3-DG: 3.46 ± 0.46 vs 1.61 ± 0.39 nmol/mg, $p < 0.01$) and kidney cortex (MG: 6.14 ± 0.59 vs 3.45 ± 0.39 nmol/mg, $p < 0.01$) were significantly elevated in HHTg rats compared to controls. Metformin treatment was associated with significantly reduced level of reactive dicarbonyls in myocardium (MG: $p < 0.05$, GL: $p < 0.05$, 3-DG: $p < 0.01$), when compared to untreated HHTg rats. There were no significant effects of metformin on dicarbonyls concentration in kidney cortex.

Conclusion: Results indicate that chronically elevated hypertriglyceridemia and FFA was associated with increased levels of methylglyoxal in serum and with markedly elevated reactive carbonyls in heart and kidney. Beneficial effect of metformin administration on reactive dicarbonyls in heart could contribute to cardioprotective effect of metformin.

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Table Serum level of sRAGE (ng/ml) in MCI and normal control patients with T2DM, Median (interquartile range)

Group	Total	Genotype			p-value ^b
		GG	GS	SS	
Total	0.53(0.39-0.98)	0.78(0.43-1.20)	0.53(0.38-0.92)	0.37(0.23-0.41)	0.012
Non-MCI	0.86(0.42-1.20)	0.95(0.43-1.95)	0.87(0.65-1.10)	0.41*	0.071
MCI	0.44(0.36-0.65)	0.47(0.41-1.01)	0.42(0.36-0.51)	0.35*	0.117
p-value ^a	<0.001	0.127	<0.001	0.554	

Data was demonstrated with interquartile range, except those marked with * was determined by mean.

^aMann-Whitney test for comparison of serum level of sRAGE between Non-MCI and MCI patients with T2DM

^bKruskal-wallis test for comparison of serum level of sRAGE between different genotypes

Abbreviations: MCI: mild cognitive impairment, sRAGE: the soluble form of receptor of advanced glycation end products, G: Gly82 allele, S: Ser82 allele

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Association between decreased serum level of sRAGE, RAGE Gly82ser polymorphism and diabetic patients with mild cognitive impairment

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Background and aims: The incidence of mild cognitive impairment (MCI) is much higher in type 2 diabetes mellitus (T2DM). As the receptor for advanced glycation end products (AGEs) and $\text{A}\beta$, RAGE was involved both in chronic complications of T2DM and Alzheimer's disease. The aim of this study was to investigate the association of the serum level of sRAGE which acts as a decoy receptor of RAGE, the Gly82ser polymorphism in RAGE gene and T2DM with MCI.

Materials and methods: A total of 109 type 2 diabetic patients, 52 subjects met the MCI diagnostic criteria proposed by the MCI Working Group of the European Consortium on Alzheimer's Disease, and 57 matched type 2 diabetic patients, were enrolled into our study. Participants were evaluated using an extensive assessment of cognitive function, serum level of sRAGE was measured by enzyme-linked immunosorbent assay, and RAGE Gly82ser polymorphism was determined by PCR-RFLP.

Results: The serum level of sRAGE in the MCI patients with T2DM (median [interquartile range] = $0.86[0.42-1.20]$ ng/ml) was significantly lower than those matched T2DM without MCI ($0.86[0.42-1.20]$ ng/ml, $p < 0.001$), even in the heterozygote group ($0.87, 0.42$ ng/ml, $p < 0.001$). It seems the 82S allele was associated with an elevated risk of MCI, the odds ratio (OR) of GS+SS is ($1.476[0.680-3.202]$), but without statistics significance because of the limitation of population size.

Conclusion: T2DM with MCI showed a lower serum concentration of sRAGE, and sRAGE may be a potential marker and preventive measures for MCI, especially in T2DM patients. The RAGE gene Gly82ser polymorphism may play a role in MCI with T2DM, but still need more research with larger population size.

OP 13 Clinical studies with GLP-1 analogues

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Liraglutide 3.0 mg reduces the prevalence of prediabetes and delays onset of type 2 diabetes in overweight/obese adults: the SCALE obesity and prediabetes trial

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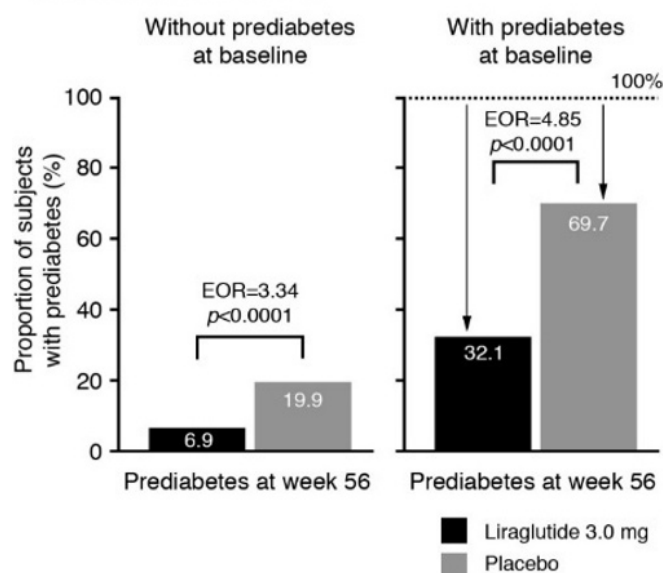
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Background and aims: This trial investigated the effects of liraglutide 3.0 mg, as adjunct to diet and exercise, on weight loss, prediabetes prevalence and onset of T2D (ADA 2010) over 56 weeks. The effects of liraglutide 3.0 mg cessation were investigated in a subsequent 12 week re-randomised period.

Materials and methods: Adults (BMI ≥ 27 kg/m² + ≥ 1 comorbidity or ≥ 30 kg/m²) were advised on a 500 kcal/day deficit diet and exercise programme and randomised 2:1 to once daily sc liraglutide 3.0 mg (n=2487) or placebo (n=1244). Randomisation was stratified by prediabetes status (ADA 2010). At week 56, individuals without prediabetes on liraglutide 3.0 mg were re-randomised 1:1 to liraglutide 3.0 mg or placebo (diet and exercise continued). **Results:** Baseline characteristics: age 45.1 yr, 78.5% female, body weight 106.2 kg, BMI 38.3 kg/m², 61.2% with prediabetes. At week 56, individuals on liraglutide 3.0 had lost 8.0% weight compared to 2.6% with placebo (estimated treatment difference [ETD] -5.4%, $p < 0.0001$, LSmeans, full analysis set-LOCF, ANCOVA). Liraglutide 3.0 mg improved fasting and post-load glycaemia compared to placebo (ETD FPG -0.38 mmol/L, PG [OGTT, area under curve] -2.02 h*mmol/L, HbA1c 0.23%-points; $p < 0.0001$ for all). Of those with prediabetes at screening, more had reverted to normoglycaemia on liraglutide 3.0 mg (69.7%) vs placebo (32.1%) at week 56. Likewise, of those with normoglycaemia at screening, more had progressed to prediabetes on placebo (19.9%) vs liraglutide 3.0 mg (6.9%) at week 56 (Fig). Few individuals developed T2D during treatment, but significantly more on placebo than on liraglutide 3.0 mg developed T2D (n=14, 1.3 events/100 patient years of exposure [PYE] vs n=4, 0.2 events/100 PYE; $p = 0.0003$). From week 56 to 68, individuals re-randomised from liraglutide 3.0 mg to placebo regained more weight (2.9%) vs those staying on liraglutide 3.0 mg (0.7%) (ETD 2.2%, $p < 0.0001$), and more individuals progressed to prediabetes on placebo (from 8.0% to 22.4%, observed means) than on liraglutide 3.0 mg (from 9.1% to 8.6%) ($p < 0.0001$). None developed T2D.

Conclusion: Consistent with the effects on body weight and glycaemia, liraglutide 3.0 mg, as adjunct to diet and exercise, was superior to placebo in reducing the prevalence of prediabetes and T2D after 56 weeks. Continued treatment was required to sustain these effects.

Figure: Proportion of subjects with prediabetes at week 56 by baseline prediabetes status



Full analysis set. Week 56 LOCF. EOR based on logistic regression. EOR, estimated odds ratio; LOCF, last observation carried forward.

Clinical Trial Registration Number: NCT01272219

Supported by: Novo Nordisk

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Effect of exenatide on postprandial cerebral and liver glucose metabolism: a double-blind randomised clinical trial

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Background and aims: The main effect of GLP-1 receptor (GLP-1R) agonists is on insulin secretion. However, since GLP-1Rs have been found in the brain it has been hypothesized that GLP-1R agonists (GLP-1RA) could improve brain glucose metabolism. We have also shown that GLP-1Rs are present in human livers and that GLP-1 injection during pancreatic clamp can reduce hepatic glucose production. The aims of the study were to evaluate the effects of a single injection of GLP-1RA Exenatide (EX) on cerebral as well as hepatic and peripheral glucose metabolism.

Materials and methods: We studied 15 male subjects with increased postprandial glucose (n=12 IGT and n=3 newly diagnosed type 2 diabetics, age=56±8 y, BMI=29±1 kg/m², HbA1c=5.7±0.1%). Each subject underwent 2 oral glucose tests (OGTT 75 g), with double blind injection of EX (5 mcg) or placebo (PLC) 30 min before OGTT; 6,6-2H-glucose was infused for 4h (2h before and 2h during OGTT) and U-13C-glucose was added to oral glucose to assess glucose absorption (RaOr), production (EGP), total rate of glucose appearance (Ra) and disposal (Rd). Brain and liver glucose uptake were measured by PET following injection of 18FDG (5mCi) at t=0 and acquiring images 1h into the OGTT.

Results: EX delayed gastric emptying (RaOr-0-60min=12.1±3.2 vs. 34.5±3.1 μmol/min-kg). Insulin response was lower in EX vs PLC (mean Insulin-0-60min 17.5±3.2 vs. 24.7±3.8 mU/l) as a consequence of lower glucose excursion (mean glucose0-60min 107±6 vs. 138±8 mg/dl, all $p < 0.02$). Despite whole body glucose Rd was comparable in EX and PLC (Rd-0-60min 10.6±0.8 vs. 11.1±0.7 μmol/min-kg), in EX we observed lower liver uptake when corrected for glucose concentrations (0.17±0.02 vs. 0.25±0.04 μmol/min-ml, $p = 0.047$) but higher when expressed as percent of RaOr ($p < 0.005$). Cerebral glucose metabolic rate (CMR) was increased after EX (0.18±0.01 vs. 0.12±0.01 μmol/min-ml; $p = 0.02$). Brain areas with the highest CMR were: Thalamus (0.23±0.02 μmol/min-ml), Occipital (0.24±0.02 μmol/min-ml) and Frontal lobes (0.19±0.02 μmol/min-ml), (all $p < 0.004$). However, in the Hypothalamus EX reduced the CMR from 0.13±0.02 to 0.09±0.01 μmol/min-ml

($p=0.003$). Total glucose CMR was inversely correlated with RaOr ($r=-0.63$; $p<0.0005$).

Conclusion: EX increased CMR in multiple areas of the brain but reduced glucose uptake in the hypothalamus. Liver glucose uptake accounted for a higher percentage of RaOr in EX. These results implicate a role of both brain and liver in the regulation of glucose metabolism by EX.

Clinical Trial Registration Number: NCT01588418

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Impact of baseline gastric emptying on effects of lixisenatide and liraglutide in type 2 diabetes mellitus (T2DM) as add-on to insulin glargine

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Background and aims: Lixisenatide (LIXI) and liraglutide (LIRA), both once-daily glucagon-like peptide-1 receptor agonists (GLP-1 RAs), were administered as add-on to insulin glargine in patients with type 2 diabetes mellitus (T2DM) for 8 wks. Here we explore whether baseline (BL) gastric emptying (GE) is a potential factor influencing the efficacy of LIXI and LIRA. **Materials and methods:** Patients were randomized 1:1:1 to once-daily s.c. LIXI 20 µg or LIRA 1.2 or 1.8 mg as add-on to insulin glargine for 8 wks. Patients fasted overnight for 10 h before a standardized ¹³C-labelled breakfast on Days -4 and 55 (281 kcal; 16% protein, 62% fat, 24% carbohydrate including 91 mg ¹³C-octanoic acid mixed with egg). ¹³C-octanoic acid breath samples were collected 30 min before breakfast (just before GLP-1 RA administration at Wk 8), every 15 min after breakfast for 2 h, then every 30 min for the next 3 h (total 15 samples). Breath samples were centrally analyzed for ¹³CO₂ by isotope-selective non-dispersive infrared spectrometry. Time for retention of ¹³C to decline to 50% ($t_{1/2}$) and time at which % of the ¹³C dose excreted/unit time reached its peak (t_{lag}) were assessed. All patients from the pharmacodynamic population were grouped into BL GE $t_{1/2}$ tertiles (T) for analysis of 8-wk outcomes: T1 92–150 min, T2 151–170 min, T3 171–334 min. Statistical analyses were performed using a linear model (ANOVA) with Bonferroni adjustment for tertile analyses.

Results: Mean (SD) BL $t_{1/2}$ and t_{lag} were 169.48 (41.07) and 113.46 (26.50) min for LIXI ($n=46$), 161.70 (23.40) and 111.23 (19.70) min for LIRA 1.2 mg ($n=44$) and 164.30 (27.13) and 109.61 (20.84) min for LIRA 1.8 mg ($n=46$). Least squares (LS) mean changes in GE $t_{1/2}$ and t_{lag} were 453.56 and 175.56 min with LIXI ($p<0.0001$ for both vs BL), 175.31 and 70.10 min with LIRA 1.2 mg, and 130.49 and 48.85 min with LIRA 1.8 mg ($p<0.05$ for all changes with LIRA vs BL). The study showed a significantly greater delay in GE with LIXI vs LIRA ($p<0.0001$) and significantly greater reductions in the AUC for postprandial glucose (AUC PPG)_{00:30-04:30 h} with LIXI ($p<0.0001$). There were no significant differences in reductions in AUC PPG_{00:30-04:30 h} and HbA_{1c} by BL $t_{1/2}$ tertile in the LIXI and LIRA groups (Table). Patients with faster BL GE experienced greater GE t_{lag} delay with LIXI (LS mean t_{lag} difference [SE] in T1 vs T3: 165.33 [44.22] min; $p=0.0009$).

Conclusion: LIXI delayed GE significantly more than LIRA. BL GE $t_{1/2}$ did not predict treatment response (AUC PPG_{00:30-04:30 h} or HbA_{1c}) with LIXI or LIRA. Delay in GE with LIXI was less pronounced in patients with slower BL GE, suggesting that risk of aggravating pre-existing GE disturbances with GLP-1 RAs is low.

Parameter	LIXI 20 µg			LIRA 1.2 mg			LIRA 1.8 mg		
	T1 (n=14)	T2 (n=15)	T3 (n=17)	T1 (n=16)	T2 (n=13)	T3 (n=15)	T1 (n=14)	T2 (n=16)	T3 (n=13)
AUC PPG _{00:30-04:30 h} , h·mmol/L									
Baseline mean (SD)	16.5 (7.1)	17.3 (6.3)	13.5 (6.6)	16.1 (4.7)	14.6 (4.6)	15.7 (7.2)	19.2 (7.3)	16.6 (5.1)	15.5 (4.6)
Wk 8 mean (SD)	3.9 (5.2)	4.1 (9.4)	2.8 (4.6)	9.5 (4.1)	9.3 (5.7)	9.8 (6.4)	8.6 (2.7)	8.6 (4.0)	9.0 (3.4)
LS mean (SE) diff vs T1	–	0.7 (2.0)	0.2 (1.9)	–	-0.3 (2.0)	0.5 (1.9)	–	0.3 (1.9)	0.9 (2.1)
LS mean (SE) diff vs T2	–	–	-0.5 (1.9)	–	–	0.8 (2.0)	–	–	0.6 (1.9)
HbA _{1c} , %									
Baseline mean (SD)	6.73 (0.40)	6.73 (0.38)	6.72 (0.41)	6.78 (0.53)	6.63 (0.33)	6.75 (0.49)	6.79 (0.39)	6.90 (0.59)	6.83 (0.30)
Wk 8 mean (SD)	6.11 (0.41)	6.34 (0.40)	6.18 (0.44)	6.10 (0.28)	6.08 (0.30)	6.23 (0.41)	6.19 (0.37)	6.10 (0.29)	6.11 (0.36)
LS mean (SE) diff vs T1	–	0.16 (0.10)	0.05 (0.10)	–	0.04 (0.10)	0.06 (0.10)	–	-0.23 (0.10)	-0.20 (0.11)
LS mean (SE) diff vs T2	–	–	-0.11 (0.10)	–	–	0.02 (0.11)	–	–	0.03 (0.10)

For AUC PPG_{00:30-04:30 h}, $n=12$ for LIRA 1.2 mg T2 and $n=13$ for LIRA 1.8 mg T1; SD, standard deviation; SE, standard error; T, tertile; $p<0.05$ for all inter-tertile statistical comparisons.

Clinical Trial Registration Number: NCT01596504

Supported by: Sanofi

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Effect of liraglutide 3.0 mg cessation on efficacy and safety/tolerability after 56 weeks' treatment in obese/overweight adults with type 2 diabetes: SCALE diabetes

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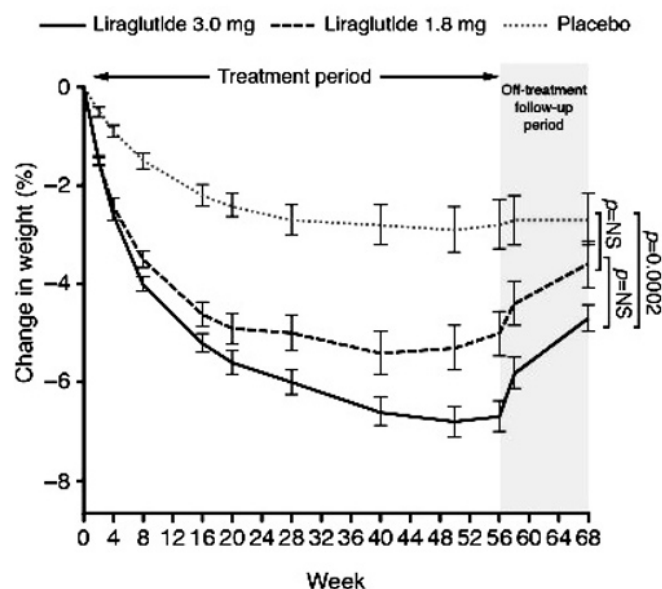
Background and aims: This was a 56-wk randomised, double-blind, placebo-controlled trial to evaluate the effect of liraglutide on weight loss induction and maintenance and glycaemic control in overweight/obese subjects with T2D. Present data are from a 12-wk follow-up period, which assessed efficacy and safety after treatment cessation.

Materials and methods: Individuals were randomised 2:1:1 to liraglutide 3.0 mg, 1.8 mg or placebo (PBO), as adjunct to diet and exercise (D&E) for 56 wks. Those who completed 56 wks' treatment entered the 12-wk off-treatment follow-up; D&E continued until wk 68. With exception of pulse, lipase, data are LS means (ANCOVA).

Results: 846 individuals (age 54.9 years, weight 105.9 kg, BMI 37.1 [27.0–67.6] kg/m², HbA_{1c} 7.9%, fasting plasma glucose (FPG) 8.8 mmol/l, 7.3 years of T2D) were randomised. More individuals on liraglutide (3.0 mg: 77%, 1.8 mg: 78%) vs PBO (66%) completed 56-wks' treatment. After 56 wks, liraglutide 3.0 mg and 1.8 mg led to greater reductions in body weight than PBO (-5.93% and -4.58% vs -1.96%, respectively; both $p<0.0001$ vs PBO); weight loss was greater with 3.0 mg vs 1.8 mg ($p=0.0024$). FPG reductions at wk 56 were greater with liraglutide 3.0 mg and 1.8 mg vs PBO (-1.89 and -1.40 vs -0.12 mmol/l, respectively; both $p<0.0001$ vs PBO), and greater with 3.0 mg vs 1.8 mg ($p=0.0061$). After liraglutide cessation weight re-gain was observed but weight loss from baseline (BL) to wk 68 remained greater with liraglutide 3.0 mg (4.66%) than PBO (-2.50%; $p=0.0002$). There was no significant difference in weight loss between liraglutide 1.8 mg (-3.70%) and PBO, nor 3.0 mg and 1.8 mg from BL to wk 68 (Fig). FPG reverted toward PBO levels within 2 wks of treatment cessation; at wk 68, change in FPG from baseline was -0.21, -0.01 and -0.12 mmol/l for liraglutide 3.0 mg, 1.8 mg and PBO, respectively ($p=NS$ for all pairwise comparisons). Mean lipase activity increased from BL to wk 56 with liraglutide 3.0 mg and 1.8 mg vs PBO (+15.3, +16.6 vs +3.7 U/l, respectively); at wk 68, lipase activity was similar in all treatment groups. Mean pulse increased from BL to wk 56 with liraglutide (2.0 and 2.1 vs -1.4 beats/min for liraglutide 3.0 mg, 1.8 mg and PBO, respectively) but decreased after treatment cessation (change from BL to wk 68: -1.3, -0.5 and 0.4 beats/min for liraglutide 3.0 mg, 1.8 mg and PBO). No adverse effects of treatment cessation on safety or binge eating were noted.

Conclusion: 12 wks after liraglutide cessation, the beneficial treatment effects on weight were reduced and effects on FPG were reversed, emphasising the need for continued treatment.

Figure: Effect of liraglutide cessation on body weight (%)



Data are observed means \pm standard error. Treatment comparisons are from an analysis of covariance model with treatment, country, sex, background treatment, baseline HbA_{1c} stratum and interaction between background treatment and HbA_{1c} stratum as fixed factors and baseline value as covariate.

Clinical Trial Registration Number: NCT01272232

Supported by: Novo Nordisk

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DURATION-1 extension: efficacy and tolerability of exenatide once weekly over 6 years in patients with type 2 diabetes mellitus

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Background and aims: In a 30-week controlled, Phase III trial (DURATION-1), exenatide once weekly (QW) exhibited greater reduction in HbA_{1c} than exenatide twice daily (–1.9% vs –1.5%; $P=0.002$), with similar weight loss, in 295 intention to treat (ITT) patients with type 2 diabetes mellitus not controlled with diet and exercise, or single or combination oral glucose-lowering therapies. Here, we report data from patients who completed 6 years of treatment, the longest assessment of the efficacy and safety of a glucagon-like peptide-1 (GLP-1) receptor agonist to date.

Materials and methods: In the open-ended extension of DURATION-1, all patients received exenatide QW.

Results: In total, 127 patients (43%) completed 6 years of treatment; baseline characteristics of these patients were similar to the ITT population. In completers, baseline [mean \pm SD] values were: HbA_{1c} $8.2 \pm 0.9\%$; fasting plasma glucose (FPG) 9.22 ± 2.26 mmol/L; weight 101 ± 17 kg; diabetes duration 7 ± 6 years. Withdrawal from the extension was most often due to withdrawal of consent (23.4%), adverse events (AEs; 5.8%) or investigator decision (5.1%). Among 6 year completers, HbA_{1c} improved significantly from baseline (least squared mean –1.6% [95% CIs –1.9, –1.4]), 45% achieved HbA_{1c} $<7.0\%$, and 32% achieved HbA_{1c} $\leq 6.5\%$. Significant improvements in FPG (–1.58 mmol/L [–2.13, –1.03]) and weight (–4.3 kg [–6.0, –2.6]) were observed, and improvements in cardiovascular markers were maintained over 6 years. Improvements in lipid levels were: total cholesterol (–0.28 mmol/L [–0.47, –0.09]); LDL cholesterol (–0.27 mmol/L [–0.42, –0.11]); HDL cholesterol (0.06 mmol/L [0.01, 0.12]); triglyceride (–0.67 mmol/L [–1.11, –0.24]). Overall, most improvements were generally observed at week 30 and main-

tained to 6 years. In the ITT population, nausea (mostly mild) was the most common AE with exenatide QW for week 0 to 30 (exposure-adjusted annual event rate [events/year of patient exposure for each period] of 0.85) but decreased over time (to 0.08 from week 30 to 6 years), as did injection site pruritus (from 0.51 to 0.02) and injection site erythema (from 0.14 to 0.01). Treatment-emergent AEs leading to withdrawal from week 30 to 6 years were infrequent (6.6%). Most common AEs leading to withdrawal were nausea and type 2 diabetes mellitus progression (loss of glucose control) ($n=2$ each). Over 6 years, 2 pancreatitis (annual rate 0.002; 1 withdrawal), 1 pancreatic carcinoma (1 withdrawal), and 3 acute renal failure cases were reported. No major hypoglycaemia was reported. Most minor hypoglycaemia occurred with concomitant sulfonylurea use.

Conclusion: In the longest extension of a Phase III GLP-1 receptor agonist study reported to date, exenatide QW was associated with significant, sustained improvements in glycaemic control and weight over 6 years in patients who continued therapy, with no unexpected safety findings.

Clinical Trial Registration Number: NCT00308139

Supported by: Bristol-Myers Squibb/AstraZeneca

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One-year efficacy and safety of IDegLira in patients with type 2 diabetes

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Background and aims: IDegLira, a combination of insulin degludec (IDeg) and liraglutide (Lira), provided the advantages and mitigated the main side effects of each of its components in a previous 26-week trial. This 26-week extension assessed whether these benefits were sustained up to 1 year of use.

Materials and methods: Patients with type 2 diabetes randomized to once-daily IDegLira, IDeg or Lira (1.8 mg), plus metformin \pm pioglitazone, continued their allotted treatment in the extension. IDegLira and IDeg were titrated to a FPG of 4–5 mmol/L (72–90 mg/dL).

Results: Of 1663 adults randomised in the main trial (mean age: 55 yr, BMI: 31.2 kg/m²), 1311 (78.8%) entered the extension: 665 (79.7%) patients on IDegLira, 333 (80.4%) on IDeg and 313 (75.4%) on Lira. Mean HbA_{1c} was reduced from baseline by 1.8% (IDegLira), 1.4% (IDeg) and 1.2% (Lira) to end of trial values of 6.4%, 6.9% and 7.1%, respectively; 78% of patients on IDegLira achieved an HbA_{1c} $<7\%$ vs. 63% for IDeg and 57% for Lira. Mean FPG was similar for IDegLira (5.7 mmol/L; 103 mg/dL) and IDeg (6.0 mmol/L; 108 mg/dL) and higher for Lira (7.3 mmol/L; 132 mg/dL). At study end, daily insulin dose was 37% lower with IDegLira (39 U) vs. IDeg (62 U). IDegLira was associated with a mean weight reduction of 0.4 kg, and had a 37% lower rate of hypoglycaemia vs. IDeg. Fewer patients had gastrointestinal adverse events with IDegLira vs. Lira (nausea: 10.3% vs. 22.3%).

Conclusion: The improved glycaemic control and more favourable safety profile seen for IDegLira in the extension trial supports the sustainability of IDegLira over at least 1 year of treatment.

Key Results: IDegLira vs. IDeg or Lira Alone				
	IDegLira vs. IDeg Estimate [95% CI]	p-value	IDegLira vs. Lira Estimate [95% CI]	p-value
HbA _{1c} change ¹ (%-points)	–0.46 [–0.57; –0.34]	<0.0001	–0.65 [–0.76; –0.53]	<0.0001
FPG change ¹ (mmol/L)	0.20 [–0.45; 0.05]	NS	–1.67 [–1.92; –1.42]	<0.0001
Weight change ¹ (kg)	–2.80 [–3.34; –2.27]	<0.0001	2.66 [2.13; 3.20]	<0.0001
Hypoglycaemia ²	0.63 [0.50; 0.79]	<0.0001	8.52 [5.09; 11.93]	<0.0001
Daily insulin dose ³ (units [U])	–23.4 [–26.4; –20.3]	<0.0001	NA	NA

¹Change from baseline to end of trial (week 52); ²Hypoglycaemia: PG <3.1 mmol/L (56 mg/dL) and/or requiring assistance; data show relative rate of events per patient year of exposure (IDegLira/IDeg); ³at week 52; Rate ratio: IDegLira/Comparator; $p<0.05$ two-sided; NA: not applicable

Clinical Trial Registration Number: NCT01336023

Supported by: Novo Nordisk

OP 14 Weight regulation and obesity

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Genetic risk score of 31 BMI loci and substantial weight change over a period of 50 years in the Malmö Diet and Cancer Study

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Background and aims: Body weight gain has increasingly become a public health threat worldwide as it is associated with many metabolic abnormalities conveying an increased morbidity and mortality. Our aim was to evaluate the contribution of genetic susceptibility defined as a genetic risk score (GRS) of 31 BMI associated single nucleotide polymorphisms (SNPs) to BMI at young, middle-age and older age, and to substantial weight gain (SWG) covering a mean time period of over 50 years starting from age of 20 years in a large Swedish cohort.

Materials and methods: Totally, 22634 non-diabetic participants (62% females) from the population based Malmö Diet and Cancer Study (MDCS) with baseline examinations 1990–1996 (age 57±8y, BMI 26±4kg/m²) and who in a questionnaire reported weight at 20 years of age (BMI 21±3kg/m²) as well as if their weight after 20 years of age had been stable, unstable, increased or decreased. A random sample of the MDCS (N=6,103), who were alive and had not emigrated from Sweden (N=4,924) were invited to a follow-up (FU) re-examination 2007–2012. Of these, 3734 subjects attended and 2676 with information of self-reported BMI from 20y were included in this study (58% females, age 73±6y, BMI 26±4kg/m², FU time from baseline 16.5±1.5y and from 20y of age 52.8±5.6y). SWG was defined as gaining (i) ≥10% of self-reported weight at 20y until baseline, (ii) ≥10% of baseline weight until FU and (iii) ≥10% of self-reported weight at 20y until FU. A weighted GRS based on 31 GWAS identified BMI susceptibility loci was created. Linear regression was used to analyze the associated effect sizes (β) per increasing quintile of GRS on BMI at 20 years of age, at baseline and at the end of FU. Further, we used logistic regression to analyze the risk (OR) per GRS quintile for belonging to the self-reported unstable weight group (23.7% of all individuals), and for SWG from 20 years age to baseline and to FU, and from baseline to FU. All analyses were adjusted for age, sex and FU time when applicable.

Results: The GRS associated with higher BMI at all ages and with highest effect size at middle-age; 20y (β 0.03±0.01, p=2.0×10⁻³⁷), baseline (β 0.23±0.02, p=1.8×10⁻³⁴) and FU (β 0.20±0.06, p=0.003). The GRS associated with 10% increased risk per GRS quintile for having unstable weight from 20y to baseline (OR 1.10 [1.07–1.13], p=1.1×10⁻⁹) and with 4% increased risk of SWG (OR 1.04 [1.02–1.07], p=0.002) from 20y to baseline. The risk increase was 42% for unstable weight reporters (p=4.1×10⁻⁷) and 15% for SWG (p=0.016) for individuals in the highest GRS quintile compared to lowest. In contrast to this, the GRS did not associate with SWG from 20y to end of FU (OR 0.97 [0.90–1.05], p=0.47) and it associated with 10% decreased risk of SWG from baseline to FU (OR 0.90 [0.84–0.97]; p=0.004) and 26% decreased risk comparing highest to lowest quintiles of GRS (OR 0.74 [0.55–1.01]; p=0.061).

Conclusion: Our data suggest that BMI GRS associates with BMI at all adult ages and with unstable weight and substantial weight gain until the later middle age. However, our results indicate inversed association with weight gain at older ages. Whether the latter can be explained by the BMI GRS accentuating the age related loss of muscle mass, other age related weight loss, age related effects on appetite regulation, or other reasons, needs to be investigated in future studies.

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Effects of genetic and environmental influences on abdominal adipose tissue compartments and hepatic lipid accumulation: a classical twin study

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Background and aims: In patients with diabetes and/or obesity, accumulation of abdominal adipose tissue and non-alcoholic fatty liver disease (NAFLD) are linked to increased cardiometabolic risk. Little is known about the genetic and environmental effects on the distribution of the abdominal adipose tissue compartments and hepatic lipid accumulation. The aim of the study was to assess the magnitude of genetic and environmental impact on the size of various abdominal adipose tissue compartments and the hepatic lipid accumulation within a cohort of healthy twin pairs.

Materials and methods: In this classical twin study, 136 adult twin subjects (58.8% women; age: 56.8±9.3 years, weight: 77.1±17.2 kg, BMI 27.3±4.9 kg/m² [x±SD], 37 monozygotic [MZ] and 31 dizygotic [DZ] pairs) were involved. The twin pairs were investigated with a 256-slice CT-scanner. A 2 mm thick axial slice was acquired at the level of L3–L4. Subsequently a 50 mm wide axial image slab was acquired below the diaphragm. For each patient CT-based measurement of waist circumference, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) quantification were performed. Liver and spleen attenuation was determined by calculating the average of three 300 mm² ROIs (regions of interest). Hepatic lipid accumulation was characterized by attenuation ratios (CT_{L/S}) and ratio of ≤0.9 was assessed as sign of NAFLD. Concordance between MZ and DZ pairs was assessed by Pearson correlations. For assessing heritability of abdominal adipose tissue compartments and that of hepatic lipid accumulation, the structural equation (A-C-E) model was used.

Results: Comparing MZ to DZ twin pairs, no significant differences were found in age (55.9±9.7 vs. 58.2±8.8 years), in BMI (27.2±3.9 vs. 26.5±4.0 kg/m²), in waist circumference (94.0±12.9 vs. 95.4±13.1 cm), in SAT (206.0±79.9 vs. 200.9±83.1 cm²), in VAT (159.9±91.0 vs. 143.0±77.6 cm²), and in CT_{L/S} ratio (1.1±0.2 vs 1.2±0.2); p>0.05 for all comparison. Strong correlations among BMI, SAT and VAT values were found in MZ twin pairs (r=0.63 [95% CI 0.34 - 0.84], r=0.74 [95% CI 0.54 - 0.90], r=0.60 [95% CI 0.34 - 0.79], respectively) whereas these correlations were weak or absent in DZ twin pairs (r=0.08 [95% CI -0.43 - 0.43], r=0.35 [95% CI 0.00 - 0.64], r=0.20 [95% CI -0.16 - 0.51], respectively). As for hepatic lipid accumulation, correlations among CT_{L/S} values were absent in both MZ pairs (r=0.30, 95% CI -0.16 - 0.67) and DZ pairs (r=0.15, 95% CI -0.15 - 0.55). Using the structural equation (A-C-E) model, relatively strong heritability index was found regarding BMI (58%, 95% CI 18–85%), SAT (74%, 95% CI 43–93%) and VAT (59%, 95% CI 22–82%) whereas environmental influences predominated in hepatic lipid accumulation (additive genetic effect 30% [95% CI 0–75%], shared environmental effect 1% [95% CI 0–41%], unique environmental effect 69% [95% CI 32–100%]).

Conclusion: Both BMI and abdominal adipose tissue compartments (SAT and VAT) have relatively strong heritability whereas hepatic lipid accumulation (presence of NAFLD) is predominantly influenced by environmental factors.

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Endogenous GLP-1 alters brain activations in response to visual food-cues in reward and satiety circuits in humans

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Background and aims: The central nervous system (CNS) plays a major role in the regulation of feeding and maintenance of body weight. Food ingestion activates the secretion of gut-hormones, such as glucagon-like peptide-1 (GLP-1). GLP-1 has been proposed to be involved in the CNS regulation of feeding, by relaying information about the nutritional status to the CNS. We

hypothesised that endogenous GLP-1 has effects on central reward and satiety circuits in overweight individuals with diabetes and healthy, lean individuals. **Materials and methods:** We included overweight patients with type 2 diabetes (T2DM) ($n=20$, mean \pm SD age 59.3 ± 4.1 yrs, BMI 32.0 ± 4.7 kg/m², 11 males) and age matched healthy, lean controls ($n=20$, mean \pm SD age 56.3 ± 6.2 yrs, BMI 22.5 ± 1.7 kg/m², 10 males). Using functional MRI (fMRI), we determined the effects of blocking endogenous GLP-1 on CNS responses to visual food-cues before and after a standardized liquid meal. To block the endogenous GLP-1 effects, intravenous administration of the GLP-1 receptor antagonist exendin 9-39 (ex9-39) was used and compared to placebo infusion. During the fMRI session, subjects were presented pictures of high calorie, low calorie and non-food objects. Imaging data were analysed using SPM8 and activation contrasts were computed (food vs. non-food).

Results: In the fasting state, obese T2DM patients versus lean individuals showed increased brain activation in response to food pictures within left amygdala, right orbitofrontal cortex and bilateral insula. The standardized meal reduced these hyperactivations in bilateral insula in patients with T2DM. Blocking endogenous GLP-1 with ex9-39 partly prevented this meal induced reduction in brain activations. In healthy lean individuals, the standardized meal also reduced activation in the insula in response to food pictures, however to a lesser extent and only in right insula. Blocking endogenous GLP-1 with ex9-39 did not prevent this meal induced reduction in brain activations.

Conclusion: Patients with T2DM showed increased brain activations in response to food pictures in areas involved in reward and satiety while fasted. Intake of a meal reduced these brain responses and this effect was blunted with blockade of endogenous GLP-1. These effects could not be detected in healthy, lean individuals. The lower brain activation in response to food pictures in healthy lean individuals at baseline, may have decreased the likelihood to detect changes in these brain activation after a meal with or without blocking endogenous GLP-1. Our findings in patients with T2DM support the hypothesis that endogenous GLP-1 is involved in the regulation of central reward and satiety.

Clinical Trial Registration Number: NCT 01363609

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A high fat diet during pregnancy and lactation affects the metabolic fate of Gpr-/- mice via hypothalamic insulin signalling and DNA-methylation of lipid metabolism genes

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Background and aims: Maternal high-fat (HF) feeding during intra-uterine (IU) and lactation (L) periods predisposes the offspring for obesity and impaired glucose homeostasis in mice. Inhibition of Glucose-dependent insulinotropic polypeptide (GIP) signaling in GIP receptor knockout mice (Gpr-/-) leads to protection from HF induced obesity. We reported that Gpr-/- mice exposed to HF during IU/L were no longer protected from diet induced obesity and had a decreased glucose tolerance in adulthood. We hypothesized that the metabolic programming of IU/L HF consumption triggers hypothalamic insulin resistance and promoter DNA methylation of key genes in fat metabolism and their subsequently altered gene expression in Gpr-/- mice.

Materials and methods: Female GIP receptor heterozygous (Gpr+/-) mice were fed either a HF (60% fat) or control (C, 10% fat) diet for 2 weeks prior to gestation and during IU/L. After weaning, male Gpr-/- and wild type (WT) offspring were kept on normal chow until the age of 25 weeks, after which all offspring were exposed to HF for the following 20 weeks. This resulted in Gpr-/- which were exposed to either a C (KO Ciu-HF) or a HF (KO HFiU-HF) diet during IU/L and a HF later in adulthood. WT mice fed a control diet during IU/L and a HF in adulthood served as controls (WT Ciu-HF). A glucose tolerance test (GTT) was performed by i.p. injection of 2 g/kg BW glucose. At 45 weeks of age, body fat content, adipocyte size and genes involved in hypothalamic insulin sensitivity and DNA-methylation of fatty acid oxidation genes in muscle were analyzed.

Results: Body fat content significantly increased in KO HFiU-HF compared to KO Ciu-HF (22.6 ± 2.5 g vs. 19.5 ± 0.8 g, respectively; $p<0.05$).

KO HFiU-HF showed significantly larger adipocytes compared to KO Ciu-HF ($p<0.005$). KO HFiU-HF and WT Ciu-HF mice had significantly higher plasma glucose levels compared to KO Ciu-HF (AUC: $3,784.5 \pm 284.9$ and $3,836.9 \pm 180.2$ vs. $2,829.5 \pm 256.3$ mmol/l*min, respectively; all $p<0.05$). In KO Ciu-HF hypothalamic gene expression of PI3K subunit p85 α was 22% down regulated compared to WT Ciu-HF mice ($p<0.01$) and back up regulated 1.27 fold in KO HFiU-HF compared to KO Ciu-HF ($p<0.05$). Expression levels of PPAR α and CPT-1 β , the key enzymes of fatty acid oxidation, were massively increased in KO Ciu-HF compared to WT Ciu-HF mice (2.45-fold for PPAR α and 1.53-fold for CPT-1 β , $p<0.01$), but then down regulated 41% for CPT-1 β and 45.2% for PPAR α in KO HFiU-HF compared to KO Ciu-HF ($p<0.05$) in muscle. One CpG-site of PPAR α and three CpG-sites of CPT-1 β showed hypomethylation in KO Ciu-HF compared to WT Ciu-HF and KO HFiU-HF. DNA-methylation was inversely correlated with gene expression.

Conclusion: Consuming a HF diet during IU/L leads to reduced central insulin sensitivity and can change the offspring's epigenetic marks resulting in decreased peripheral fatty acid oxidation, which reverses the protection of diet induced obesity in Gpr-/- mice.

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Weight loss normalises lowered mu-opioid receptor availability in the morbidly obese

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Background and aims: Neurochemical pathways involved in overeating and obesity are poorly understood. Our previous positron emission tomography (PET) studies suggest that morbidly obese versus normal-weight human subjects have lower mu-opioid receptor (MOR) availability but unaltered dopamine D2 receptor (D2R) availability in the brain. Investigating the effects of weight loss could reveal whether altered receptor availability is a state or a trait of obesity.

Materials and methods: We recruited 21 morbidly obese women (mean BMI 41, mean age 42), with nine having type 2 diabetes (T2DM), eligible for bariatric surgery, and measured their brain D2R availability using PET with [¹¹C]raclopride and MOR availability with [¹¹C]carfentanil before and six months after the bariatric surgery. 14 non-obese age-matched healthy women (mean BMI 23, mean age 45) formed the control group. Receptor availability was assessed as the binding potential (BPND). Both ROI-based statistics and statistical parametric mapping were used to compare the parametric BPND maps within subjects (pre- vs. postoperative).

Results: Average weight loss after surgery was 25 kg. T2DM recovered in six subjects. Weight loss normalized lowered [¹¹C]carfentanil BPND in the morbidly obese, with on average 31 % higher MOR binding in the postoperative scan (Figure 1). Significant changes were observed in several areas implicated in reward processing, including ventral striatum, dorsal caudate, insula, amygdala, thalamus and anterior cingulate cortex ($ps < 0.005$). However, weight loss did not influence [¹¹C]raclopride binding in any brain region (Figure 1). Glycaemic status did not affect the receptor density before or after weight loss.

Conclusion: Endogenous opioid system plays an important role in the pathophysiology of obesity while the role of dopaminergic pathways remains questionable. Because bariatric surgery and concomitant weight loss recovers MOR availability, lowered MOR availability is a consequence of obesity and may mediate maintenance of excessive energy uptake. Understanding the opioidergic contribution to overeating is thus critical for developing new treatments for obesity.

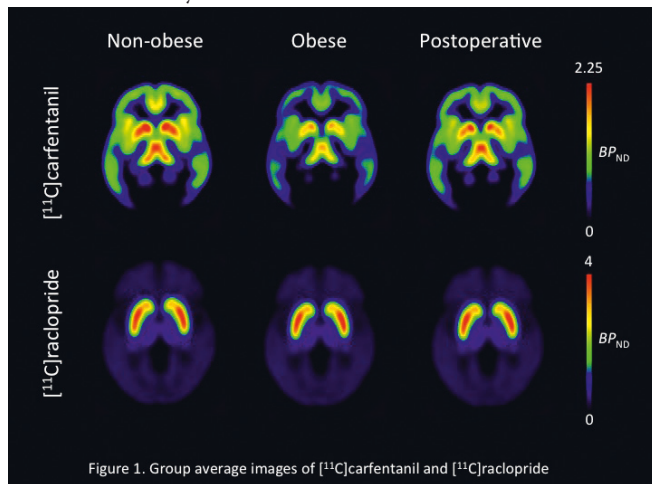


Figure 1. Group average images of [^{11}C]carfentanil and [^{11}C]raclopride

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KBP-042 lowers body-weight and sustains weight-loss in high fat-diet rats

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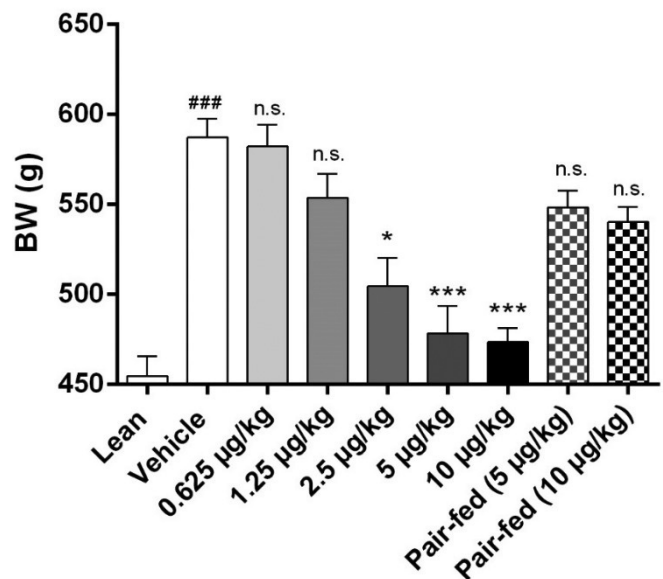
Background and aims: KBP-042 is a dual amylin- and calcitonin receptor agonist, with superior activity compared to salmon calcitonin. In this study we evaluated the long term potential of KBP-042 as a treatment against obesity. We evaluated body weight, adiposity, glucose tolerance and insulin action in a rat model of obesity.

Materials and methods: Male sprague-Dawley rats were fed a high fat-diet for ten weeks resulting in an obese and glucose intolerant phenotype. Based on body weight the rats were randomized into the treatment groups: Vehicle (saline), 0.625 $\mu\text{g}/\text{kg}$, 1.25 $\mu\text{g}/\text{kg}$, 2.5 $\mu\text{g}/\text{kg}$, 5.0 $\mu\text{g}/\text{kg}$, 10.0 $\mu\text{g}/\text{kg}$ KBP-042 s.c. once daily ($n=10$), as well as pair-fed controls for the two highest concentration groups (pair-fed 10 $\mu\text{g}/\text{kg}$ and pair-fed 5 $\mu\text{g}/\text{kg}$) which were food restricted to match the food intake of the 10 $\mu\text{g}/\text{kg}$ and 5 $\mu\text{g}/\text{kg}$ groups.

Results: A dose-dependent and sustained weight-loss was obtained. For the KBP-042 10 $\mu\text{g}/\text{kg}$ group the treatment resulted in a 4.7 % weight reduction (20 % vehicle-corrected), while only a transient change in food intake. Furthermore, while the pair-fed groups did not lower body weight, they did not match the extent of their corresponding treatment group, +5.6 % and +7.1 % respectively (-5.6 % and -8.0 % vehicle-corrected). Adiposity was vastly improved after treatment with KBP-042. The visceral fat depots were significant reduced (~40% perirenal AT: $p<0.01$ 10 $\mu\text{g}/\text{kg}$ vs. vehicle, ~33% epididymal AT $p<0.05$ 10 $\mu\text{g}/\text{kg}$ vs. vehicle), and the subcutaneous inguinal AT was significantly reduced (~35% $p<0.05$ 10 $\mu\text{g}/\text{kg}$ vs. vehicle) all depots were lowered in a dose-dependent manner. For all the depots that we measured, we observed no difference between the pair-fed groups and vehicle. The treatment groups showed improved glucose tolerance both in the oral glucose tolerance test (OGTT) (21 % $p<0.001$ vehicle vs. 10 $\mu\text{g}/\text{kg}$ and 17 % $p<0.001$ vehicle vs. 5 $\mu\text{g}/\text{kg}$) and in the intravenous glucose tolerance test (IVGTT) (10 % $p<0.02$ vehicle vs. 10 $\mu\text{g}/\text{kg}$) without increasing insulin levels. Furthermore, the incretin GIP was significantly lowered after oral glucose load for the 2.5 $\mu\text{g}/\text{kg}$, 5 $\mu\text{g}/\text{kg}$ and 10 $\mu\text{g}/\text{kg}$ groups ($p<0.01$ vs. vehicle) while there were no changes in the pair-fed groups.

Conclusion: In conclusion KBP-042 induced and sustained a significant and dose-dependent weight-loss, reduced adiposity and improved the glucose tolerance in high-fat diet rats, independent of calorie-restriction. This study demonstrates KBP-042 as a therapeutic agent against obesity in an intervention model of obesity.

Body Weight (day 56)



OP 15 Diabetic nephropathy: epidemiology and genetics

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Prevalence of nonalbuminuric chronic kidney disease (CKD) is increasing in patients with type 1 diabetes mellitus

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Background and aims: According to traditional paradigms of diabetic nephropathy, albuminuria precedes glomerular filtration rate (GFR) loss in the progression to CKD. However, even in type 1 DM (T1DM), recent findings (DCCT/EDIC) demonstrate that GFR loss may occur in normoalbuminuria. We compared prevalence of different CKD phenotypes in two cohorts of Italian T1DM subjects.

Materials and methods: The first cohort (C1) consisted of 777 T1DM recruited 2001–2009 at our Metabolic Unit; the second cohort (C2) consisted of 936 T1DM belonging from the EURODIAB IDDM Complications Study (they attended 9 Italian centres in 1989–1991). Eligibility criteria were the same for both cohorts. However, C2 was stratified at enrollment by sex, age (15–29, 30–44, 45–59 yrs) and duration (1–7, 8–14, ≥15 yrs). Methods employed to assess diabetic complications were similar.

Results: Gender distribution (M/F: 52/48% vs 51/49%) and current smokers (29.6% vs 30.4%) were similar. Due to inclusion criteria, C1 were older (40.2±11.7 vs 32.1±10.5) and had longer diabetes duration (19.4±12.2 vs 14.4±9.0 years; both $p<0.0001$). C1 had higher BMI, sBP and hypertension rate (35.2% vs 21.9%), lower total- and LDL-C (higher rate of lipid-lowering treatment: 12.9% vs 2.4%), higher HDL-C ($p<0.0001$ for all), with no differences in triglycerides. HbA1c was slightly lower in C1 (7.83±1.17 vs 8.08±1.79%, $p=0.0008$). C1 were more frequently on BP-lowering drugs (19.4% vs 9.4%) and RAS blockers (17.5% vs 6.7%, both $p<0.0001$). Prevalence of any retinopathy was similar (41.3% vs 41.2%), while proliferative retinopathy was more frequent in C1 (15.6% vs 8.8%, $p<0.0001$), with no difference in CV events (8.5% vs 9.9%). Albuminuria was more frequent in C2: rates of normo- (nA, ACR <30 mg/g), micro- (ACR 30–299) or macro- (ACR ≥300) were 91.6, 6.4 and 1.9% in C1, and 79.2, 14.1 and 6.7% in C2 ($p<0.0001$). Stage 1 (≥90), 2 (60–89) and ≥3 (<60 ml/min/1.73m², MDRD) eGFR were 57.3, 39.0 and 3.7% in C1, and 84.8, 13.2 and 1.9% in C2 ($p<0.0001$). In C1, 89.4% had no-CKD; 6.8% stages 1–2 and 3.7% stages ≥3 CKD; in C2, 78.6, 19.5 and 1.9%, respectively ($p<0.0001$). The albuminuric (Alb+) and non-albuminuric (Alb-) phenotypes were present in 41.4 and 58.6% of stages ≥3 in C1 vs 72.2 and 27.8% in C2 ($p<0.039$). In both cohorts, nA was splitted in “normal albuminuria” (ACR <10 mg/g) and “low-microalbuminuria” (ACR 10–29 mg/g); stages 1–2 was stratified in 2a (75–89) and 2b (60–74 ml/min/1.73m²). Also in 2b eGFR, nA was common and more frequent in C1 than C2 (88.7% vs 67.5%, $p=0.006$). Considering 2b and ≥3 CKD as a whole, nA was present in 80% of C1 and 55% of C2 ($p=0.001$); “normal albuminuria” in 60 and 29%, respectively ($p=0.0001$).

Conclusion: Nonalbuminuric renal function impairment is detectable in a high proportion of T1DM. Thought several factors may contribute to explain the differences in the rate nonalbuminuric CKD phenotypes between the two cohorts, the possibility of really increasing incidence of the nonalbuminuric phenotypes, likely driven by improvements in care, cannot be ruled-out.

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Determinants of urinary albumin excretion within the normal range in patients with type 2 diabetes from the RIACE cohort

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Background and aims: Higher values of albumin excretion rate (AER) within the normoalbuminuric range are known to correlate with higher cardiovascular disease (CVD) and renal risk. This cross-sectional analysis was aimed at assessing the determinants of AER in normoalbuminuric subjects with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study.

Materials and methods: The RIACE cohort consists of 15,773 patients, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007–2008. Exclusion criteria were dialysis or renal transplantation. AER was measured by immunonephelometry or immunoturbidimetry and eGFR was calculated by the MDRD study and the CKD-EPI equation. The 11,538 subjects with normoalbuminuria (73.2% of the entire cohort) were stratified in those with normal albuminuria (NA, AER<10 mg/24h; n=6,023, 52.2%) and low albuminuria (LA, AER=10–29 mg/24h, n=5,515, 47.8%).

Results: Compared with NA subjects, LA patients were more frequently males and former or current smokers, had longer diabetes duration, higher HbA_{1c}, diastolic blood pressure, and, in men only, BMI waist circumference, triglycerides, and LDL-cholesterol (in men only), and higher prevalence of family history of hypertension, use of antihypertensive drugs and oral hypoglycaemic agents (OHA) or insulin, hypertension, and the metabolic syndrome. Moreover, patients with LA had higher prevalence of: non-advanced (12.1% vs. 9.9%) and advanced (7.6% vs 6.5%) retinopathy ($p<0.0001$); any CVD (21.9% vs. 17.9%, $p<0.0001$); myocardial infarction (10.6% vs. 9.3%, $p=0.019$); and coronary (15.0% vs. 12.5%, $p<0.0001$ in males only) and peripheral (4.7% vs. 3.6%, $p=0.003$ in females only) artery disease. eGFR correlated significantly with AER ($p<0.0001$), though prevalence of LA increased only from category 3b for eGFR_{MDRD} and 3a for eGFR_{CKD-EPI}. Logistic regression with backward variables selection showed an independent correlation of LA with age (OR=1.018), smoking status (former, OR=1.158; current, OR=1.234), HbA_{1c} (OR=1.065), triglycerides (OR=1.001), diastolic BP (OR=1.010), waist circumference (OR=1.004), use of RAS blockers (OR=1.081) or DHP Ca-channel blockers (OR=1.178), use of OHA (OR=1.324), OHA+insulin (OR=1.378), or insulin alone (OR=1.535), and family history of hypertension (OR=1.321).

Conclusion: Several factors that are potentially amenable of intervention are associated with an early increase of AER within the normoalbuminuric range in patients with type 2 diabetes from the RIACE cohort.

Clinical Trial Registration Number: NCT00715481

Supported by: Fo.Ri.SID, DEM, Eli-Lilly, Takeda, Chiesi, Boehringer-Ingelheim

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Folkhälsan Research Foundation, the Wilhelm and Else Stockmann Foundation.**Kidney injury molecule - 1 is linked to the loss of kidney function and life span, in patients with type 1 diabetes**

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Background and aims: We previously showed that kidney injury molecule 1 (KIM-1) predicts progression to ESRD, but its relation with kidney function and mortality in patients with type 1 diabetes is not very clear. The aim of this study is to investigate if KIM-1 predicts and has a causal role in the loss of kidney function or life span by a Mendelian randomisation approach.

Materials and methods: We enrolled at baseline 1573 patients with type 1 diabetes divided in three groups: 953 patients with normal AER, 269 patients with microalbuminuria and 350 patients with macroalbuminuria. KIM-1 was measured at baseline, by ELISA and normalized with urinary creatinine. Kidney function was evaluated by estimated GFR according to CKD-EPI formula. Life span was considered the vital time. The predictive value of KIM-1 for the loss of kidney function and life span was evaluated by linear regression. Multiple linear regression models estimated the observed effect (association) of KIM-1 on eGFR or life span. We assessed the causal effect of KIM-1 on eGFR and life span, by Instrumental variable analysis, using two stage least squares method (2SLS), with the top SNP associated with KIM-1 from our GWAS.

Results: In linear regression analysis KIM-1 predicted the loss of GFR in univariate analysis ($\beta = -4.522$; $P < 0.0001$), but not when adjusted for albumin excretion rate (AER) ($\beta = 0.336$; $P = 0.70$). Also KIM-1 predicted the life span in univariate linear regression analysis ($\beta = 0.812$; $p = 0.006$), adjusted for AER ($\beta = 1.103$; $P < 0.0001$), but not eGFR ($\beta = 0.318$; $P = 0.26$). In our GWAS the single nucleotide polymorphism - rs2036402 presented the strongest association with KIM-1 ($p = 3.54 \times 10^{-38}$). The instrumental analysis (IV), showed that increased KIM-1 was associated with decreased eGFR, even after adjusting for AER ($\beta = -5.981$; $P = 0.021$). Furthermore, the IV analysis showed that increased KIM-1 was associated with life span even adjusted for AER ($\beta = 1.920$; $p = 0.038$), but not when adjusted for eGFR ($\beta = 1.167$; $p = 0.19$).

Conclusion: In summary, increased KIM-1 levels is associated with decreased eGFR in patients with type 1 diabetes. Furthermore KIM-1 is associated with life span, in the same patients and this association seems to be mediated by eGFR.

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A novel podocyte gene, R3h domain containing-like inhibits non-canonical TGF-beta signalling

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Background and aims: Diabetic nephropathy (DN) is a major microvascular complication of diabetes mellitus and is one of the most common causes of end-stage renal disease. However, the molecular mechanisms underlying DN remain largely unknown. We previously identified >300 glomerulus-enriched transcripts, including R3h domain containing-like (R3hdm1), through large-scale sequencing and microarray profiling of the mouse glomerular transcriptome. Therefore, the aim of this study was to analyze both the in vivo and in vitro functions of R3hdm1.

Materials and methods: R3hdm1 mRNA expression was examined in mice by non-radioactive in situ hybridization, and protein expression was evalu-

ated by immunohistochemistry (IHC) or western blot (WB) analysis. Cultured murine podocytes, R3hdm1 knockout (R3hKO) podocytes, and human fibroblasts overexpressing R3hdm1 were used for in vitro analysis. The effects of transforming growth factor- β (TGF- β) on R3hdm1 expression and function were evaluated by real-time polymerase chain reaction and WB analysis. R3hdm1 knockout mice were generated by homologous recombination. Diabetes was induced in mice by intraperitoneal injection of streptozotocin (STZ).

Results: Both R3hdm1 mRNA and protein were specifically expressed in glomerular podocytes. TGF- β has been reported to play a major role in DN. Therefore, we evaluated the effects of TGF- β on R3hdm1 expression and function. TGF- β can activate not only Smad-dependent pathways but also non-Smad pathways, including the non-canonical p38 mitogen-activated protein kinase (MAPK) pathway. When human fibroblasts were treated with TGF- β , phosphorylation of p38 MAPK (pp38MAPK) increased by 2.26 ± 0.15 -fold (mean \pm SEM). On the other hand, TGF- β -induced pp38MAPK expression was significantly (54%) reduced in human fibroblasts overexpressing R3hdm1. Smad phosphorylation was independent of R3hdm1, indicating that R3hdm1 inhibited the non-Smad pathway but not the Smad-dependent pathway. Furthermore, TGF- β increased pp38MAPK by 2.35 ± 0.01 -fold in primary cultured podocytes. TGF- β -induced pp38MAPK was significantly higher (2.03-fold) in R3hKO podocytes than in the wild-type (WT) controls. R3hKO mice showed aberrant podocyte structure and partial thickening of the glomerular basement membrane. Furthermore, IHC revealed that pp38MAPK was increased by 2.14 ± 0.490 -fold in R3hKO glomeruli compared with the WT controls. R3hdm1 mRNA expression in the glomeruli was increased by 2.7 ± 0.73 -fold in the diabetic mice compared with the WT controls. Finally, we induced diabetes in both the WT and R3hKO mice by STZ injection and found that the prevalence of albuminuria was significantly increased in the diabetic and R3hKO mice compared with the diabetic WT controls.

Conclusion: We identified a novel podocyte-specific gene, R3hdm1, which is regulated by TGF- β signaling. This gene product inhibits TGF- β -induced p38 MAPK signaling. Our results suggest that podocyte-specific therapy presents a viable option to inhibit DN in the near future.

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ABCG8 polymorphisms and renal disease in type 2 diabetic patients

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Background and aims: The ATP-binding cassette transporters G5 and G8 (ABCG5 and ABCG8) play an important role in the intestinal sterol absorption and biliary acid secretion. They are involved in the elimination of plant sterols. Polymorphisms of the genes coding for these transporters have been involved in absorption of sterols, cholesterol synthesis, gallstone disease, insulin resistance and cardiovascular risk. Lipid metabolism and insulin resistance are associated with diabetic nephropathy. The aim of our study was to assess the associations between two ABCG8 coding polymorphisms, T400K and D19H, and the incidence of renal events in type 2 diabetic subjects.

Materials and methods: First, participants were the 3,123 French type 2 diabetic subjects with micro- or macro-albuminuria from the genetic substudy of the DIABHYCAR trial. These participants had serum creatinine concentrations ≤ 150 μ mol/l. The drug tested against placebo was low-dose ramipril (1.25 mg/day). The mean duration of follow-up was 4 years. Renal events were defined as a doubling of serum creatinine concentration or end-stage renal disease at follow-up. This trial showed no effect of the drug on the incidence of renal events. We then used a second population (DIAB2NEPHROGENE/SURDIAGENE study) of 2,452 patients with type 2 diabetes for the purpose of replication. Polymorphisms T400K and D19H were genotyped using Kasper method. The genotyping success rate was > 98%.

Results: Seventy-five renal events (66 doublings of serum creatinine concentration, and 9 cases of end-stage renal failure) occurred in genotyped patients during the study. The 400K allele was significantly associated with a higher risk of incident renal event: sex and age adjusted OR 1.66, 95%CI 1.15-2.39, $P = 0.007$. This association was still significant after multiple additional adjustments for values at baseline (BMI, blood lipids, estimated glomerular filtration rate, urinary albumin excretion): OR 1.57, 95%CI 1.07-2.31, $P = 0.02$. There was a trend toward an interaction with ramipril treatment (P interaction = 0.06). The 400K allele was associated with a higher risk in the ramipril

treated group (OR 2.41, 95%CI 1.41–4.13, $P < 0.001$) but not in the placebo group (OR 1.21, 95%CI 0.72–2.03). In the replication cohort, no association was found with the incidence of new renal events, but the 400K allele was associated with the prevalence of end stage renal disease at baseline (sex, age, BMI adjusted OR 1.77, 95%CI 1.01–3.10, $P = 0.045$). No significant association was found between the D19H polymorphism and the risk of diabetic nephropathy.

Conclusion: A polymorphism of the sterol transporter ABCG8 has been associated with prevalence of end stage renal disease and with the incidence of new renal events in type 2 diabetic patients. These results described for the first time should be replicated.

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Genome-wide association studies of diabetic kidney disease

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Background and aims: Diabetes mellitus is associated with devastating chronic complications including diabetic kidney disease (DKD), a leading cause of end-stage renal disease (ESRD). The risk of developing DKD is partly determined by genetic factors. In the SUMMIT consortium we performed meta-analysis of genome-wide association studies in European T1D and T2D patients to identify genetic determinants of DKD. The genetic variants regulating early stages of disease may differ from those that determine progression to severe disease. We therefore analyzed microalbuminuria (MiAU), reflecting early pathologic changes and endothelial dysfunction, and more severe DKD, i.e. macroalbuminuria (MaAU), chronic kidney disease (CKD) and ESRD, separately.

Materials and methods: DKD was defined as MiAU, MaAU or ESRD, whereas controls had normoalbuminuria and diabetes duration > 10 years. The study included four cohorts of T2D patients: GoDARTS (n=3240), SDR (n=1830), Steno (n=294) and MNI (n=353). We analyzed ~9.2 million single nucleotide polymorphisms (SNPs), imputed based on the 1000G (March 2012) reference panel, using logistic regression, adjusting for sex, age at onset and duration of diabetes, for five phenotypes: DKD, CKD, MiAU, MaAU+ESRD and ESRD. Meta-analyses were performed using a fixed effects model. We also performed joint analyses with four T1D studies analyzed using similar methods and phenotype definitions: FinnDiane (n=3415), Eurodiab (n=789), SDR (n=556) and Cambridge (n=396).

Results: In T2D, rs2206136 (OR=1.2, $p = 2.1 \times 10^{-8}$) near *PLCB4* was significantly associated with CKD (3094 cases, 2906 controls). Nominal associations were also seen with DKD, MiAU and ESRD ($p < 0.01$). The strongest associations for other phenotypes were rs183249293 (OR=0.42, $p = 2.4 \times 10^{-7}$) for DKD (3345 cases, 2372 controls); rs2150814 (OR=0.8, $p = 8.1 \times 10^{-8}$) near *GABRR1* for MiAU (1989 cases, 2238 controls); rs76262407 (OR=5.5, $p = 1.1 \times 10^{-7}$) for ESRD (371 cases, 4471 controls); and rs116354014 (OR=4.5, $p = 2.5 \times 10^{-7}$) for MaAU+ESRD (1339 cases, 2372 controls). In the joint T1D+T2D meta-analysis rs2331712 near *STXBP5L* reached genome-wide significance (OR=1.4, $p = 1.9 \times 10^{-8}$, 1184 cases, 8466 controls) for ESRD. The effect size was slightly higher in T2D (OR=1.8, $p = 4.1 \times 10^{-6}$) compared to T1D (OR=1.3, $p = 0.0008$). No association was seen with MiAU or CKD ($p > 0.1$) suggesting an effect specifically on late stages of disease.

Conclusion: We have identified two new candidate loci for DKD situated near the *PLCB4* (phospholipase C, beta 4) and *STXBP5L* (syntaxin binding protein 5-like) genes. *PLCB4* has previously been shown to be differentially expressed in DKD, making it a strong candidate gene, possibly affecting disease risk via the DAG/PKC pathway.

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OP 16 Mechanisms of cardiovascular disease

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Low levels of C-peptide production protect from complications and improve HbA_{1c} control in longstanding type 1 diabetes

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Background and aims: Low levels of C-peptide (2.5–50 pmol/L) are produced for decades after the onset of type I diabetes, but the clinical significance is unknown. This study seeks to understand the clinical significance of extremely low levels (< 10 pmol/L) of C-peptide production.

Materials and methods: We evaluated the relationship between low levels of C-peptide and age of onset (n=1273), diabetes complications (n=324), HbA_{1c} control (n=807), risk of hypoglycemia (n=331), and response to a mixed meal tolerance test (MMTT, n=9) in patients with longstanding type I diabetes. C-peptide samples were tested using a regular or ultrasensitive C-peptide ELISA kit. Hypoglycemia risk was determined using a validated survey. For the MMTT, each subject had three MMTT tests performed over a three-month period to understand biological variation in stimulated C-peptide at low ranges of basal pancreas function.

Results: After adjusting for disease duration, we found that extremely low levels of C-peptide were associated with risk for diabetes-related complications (e.g., nephropathy, neuropathy, cardiovascular disease) ($p = 0.03$) and poorer metabolic control captured by HbA_{1c} ($p = 0.01$). There was no association between C-peptide < 10 pmol/L and risk of hypoglycemia. Even at extremely low levels of C-peptide production, beta-islet cells responded to a glucose challenge from a MMTT by secreting insulin, indicating that pancreatic function is preserved, but patients with undetectable C-peptide (<1.5–2.5 pmol/L) did not respond to the MMTT.

Conclusion: Extremely low levels of C-peptide appear to have clinical significance and may be helpful in defining groups of long term diabetics who are at risk for complications or poor metabolic control.

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Skin AGE fluorophore LW-1 predicts micro- and subclinical macrovascular complication progression in type 1 diabetes

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Background and aims: Skin collagen Long Wave autofluorescence (LW) is widely used as a surrogate marker for the accumulation of advanced glycation end-products (AGE). We determined the relationship between LW-1, a novel fluorescent skin collagen marker of partially known structure, with glycaemia, other collagen AGEs and the severity of complications in biopsies obtained from 216 participants at closeout of the Diabetes Control and Complications Trial (DCCT) study in 1993.

Materials and methods: Skin biopsy proteolytic digests were prepared as described and LW-1 content was determined by fluorescence HPLC.

Results: LW-1 levels increased with age and diabetes duration and when corrected for these parameters, they were significantly decreased with intensive vs. conventional diabetes therapy in both primary and secondary DCCT cohorts (P less than 0.0001). They were associated with retinopathy progression (sustained greater than 3 microaneurysms ever in DCCT) and albumin excretion rate (AER closest to biopsy > 40 mg/24 hr) ($P = 0.0038$) which remained significant after adjustment for DCCT HbA_{1c}. In EDIC, LW-1 correlated with retinopathy progression at EDIC Year 13–16, intima media thickness (IMT) at Yr 6 (n=147, $P = 0.014$) and left ventricular mass (EDIC Yr 14–16) adjusted for EDIC A1c ($P = 0.004$). LW-1 correlated highly (P less than 0.0001) with collagen modifications in the order glucosepane > pento-

sidine > pepsin insolubility > collagen fluorescence > MG-H1, and weaker with fructose-lysine ($P = 0.0021$), and explained almost 10% of the variability in DCCT HbA1c.

Conclusion: LW-1 is a novel robust marker which predicts progression of retinopathy and nephropathy and future progression of IMT and LVM increase independently of the effects of HbA1c.

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Plasma levels of MMP-2, -3 and -10, and of TIMP-1 are associated with vascular complications in patients with type 1 diabetes: the EURODIAB prospective complications study

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Background and aims: Extracellular matrix remodeling by matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMP) may lead to micro- and/or macrovascular complications in type 1 diabetes. Evidence so far with regard to the associations between plasma MMPs and vascular complications is contradictory, as both positive and null associations have been described in relatively small studies. Therefore we have investigated the associations between plasma MMP-1, -2, -3, -9 and -10, and TIMP-1 on the one hand and cardiovascular disease (CVD) and microvascular complications on the other in a cohort of type 1 diabetic patients, and the extent to which such associations may be explained (i.e. mediated) by low-grade inflammation (LGI) and/or endothelial dysfunction (ED), as estimated by plasma markers.

Materials and methods: The study included 493 type 1 diabetic patients (39.5 ± 9.9 years old, 51% men) from the EURODIAB Prospective Complications Study. We used linear regression analyses to investigate the differences in plasma levels of MMP-1, -2, -3, -9 and -10, and of TIMP-1 between patients with vs. without CVD, albuminuria (normo, micro and macro) and retinopathy (no, non-proliferative and proliferative). All analyses were adjusted for age, sex, duration of diabetes and HbA1c and additionally for other cardiovascular risk factors and other vascular complications, as appropriate. Standardized concentrations of plasma CRP, IL-6 and TNF- α were averaged to compose an LGI score and standardized concentrations of plasma sVCAM-1 and sE-selectin composed an ED score. These LGI and ED scores were then added to the fully adjusted model.

Results: Patients with CVD ($n=118$) had significantly higher levels of TIMP-1 [$\beta = 0.32$ SD (95%CI 0.12; 0.52)] than those without CVD ($n=375$). Higher plasma levels of MMP-2, MMP-3, MMP-10 and TIMP-1 were associated with increasing levels of albuminuria (p -trends were 0.034, 0.004, 0.004 and 0.001, respectively). The severity of retinopathy was significantly associated with higher levels of MMP-2 (p -trend=0.022). MMP-1, -3, -9 and -10, and TIMP-1 were significantly and positively associated with the LGI score. MMP-2, MMP-10 and TIMP-1 were significantly and positively associated with the ED score. Nevertheless, the significant associations between plasma levels of MMPs and TIMP-1 on the one hand and CVD, albuminuria and retinopathy on the other were largely independent of LGI and ED.

Conclusion: In patients with type 1 diabetes, prevalent CVD, albuminuria and retinopathy were significantly associated with higher plasma levels of MMP-2, MMP-3, MMP-10 and TIMP-1, and these associations were largely independent of LGI and ED. Thus, these data support the hypothesis that extracellular matrix remodeling, by the action of MMPs and TIMP-1, is involved in vascular complications in type 1 diabetes.

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Acute glucose and insulin changes during OGTT relate to LV-myocardial deformation changes, untwisting and coronary-flow-reserve through increased arterial stiffness

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Background and aims: Insulin resistance is linked to endothelial dysfunction and increased arterial stiffness. Increased Arterial stiffness may impair LV function. We investigated whether first-degree relatives of diabetic patients have similarly impaired coronary microcirculation, LV-myocardial strain and twisting with diabetic patients, as assessed after an oral glucose tolerance test (OGTT).

Materials and methods: In 76 subjects without known diabetes a standard 75-gr OGTT was performed. Glucose and insulin levels, pulse wave velocity (PWVa) and augmentation index (AI) (Arteriograph, Tensiomed) were measured at 0, 30, 60, 90 and 120 min after glucose load. At 0 and 120 min, we measured: a) E' and A' mitral annular velocities and their ratio E'/A' using tissue Doppler imaging, b) LV longitudinal (GLS-%), strain, systolic (LGSr) and diastolic strain rate (LGSrE), twisting (Tw -deg), peak twisting (Tw-deg/sec) velocity, and peak untwisting (unTw) velocity using speckle tracking echocardiography and c) coronary flow reserve (CFR) of the LAD after adenosine infusion using Doppler echocardiography. We assessed insulin resistance using insulin sensitivity index (ISI) which includes both insulin and glucose levels at baseline and 120 min after OGTT.

Results: Of the 76 subjects, 36 who were first degree relatives of diabetics had normal OGTT (relatives), 20 had normal OGTT and no family history of diabetes (normals), and 20 had abnormal OGTT (diabetics). Age, sex and BMI were similar between subgroups ($p=ns$). Compared to normals, diabetics and relatives had both higher baseline PWVa (9.3 ± 2 vs. 8.1 ± 2 vs. 7.2 ± 1.6 m/sec), AI (23 ± 9 vs. 24 ± 14 , $18 \pm 15\%$), insulin (median 14 vs. 15 vs. 10 μ U/ml, $p < 0.09$), and lower ISI (50 ± 24 vs. 73 ± 22 vs. 93 ± 17), baseline E'/A' (0.7 ± 0.2 vs. 0.98 ± 0.2 vs. 1.1 ± 0.3), LGSr (-0.95 ± 0.1 vs. -0.94 ± 0.1 vs. -1.1 ± 0.15 l/sec), LGSrE (0.98 ± 0.1 vs. 1.1 ± 0.1 vs. 1.3 ± 0.15 l/sec, $p < 0.05$) Tw (15 ± 7 vs. 13 ± 5 vs. 17 ± 7) and unTw velocity (-95 ± 31 vs. -94 ± 40 vs. 116 ± 36) and lower CFR (2.7 ± 1.1 vs. 2.6 ± 0.9 vs. 3.0 ± 0.6) ($p < 0.05$ for all comparisons). Compared to baseline, insulin was increased at 120min, to 87μ U/ml (521%) in diabetics, 59μ U/ml (293%) in relatives and 29μ U/ml (190%) in normals ($p < 0.05$). PWVa was increased at 120min to 8.9 ± 2 m/s (10%) in relatives, was reduced to 6.8 ± 2 m/s (6%) in normals and remained high (9.4 ± 2 m/s) in diabetics ($p < 0.05$). Tw and unTw velocity at 120min, was increased to 17 ± 7 (13%), and -105 ± 31 (10%) in diabetics, to 15 ± 5 (15%) and -105 ± 40 (10%) in relatives and was reduced to 13 ± 7 (24%) and -87 ± 30 (25%) in normals ($p < 0.05$). CFR was decreased to 2.4 ± 0.7 (14%) in diabetics, 2.3 ± 0.7 (12%) in relatives and 2.8 ± 0.9 (6%) in normals ($p < 0.05$). ISI, insulin and glucose at 120 min were related with PWV, CFR, GLS, LGSr E, Tw, Tw velocity unTw velocity and E'/A' in both diabetics and relatives ($p < 0.05$). At 0 and 120 min, PWV was related with the corresponding LGSr E, Tw, Tw velocity unTw velocity and E'/A' ($p < 0.05$).

Conclusion: Acute hyperglycaemia and hyperinsulinemia post OGTT are related to abnormal LV-myocardial deformation, twisting and untwisting possibly through increases in arterial stiffness and impairment of coronary microcirculatory function in first degree relatives and diabetics

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Vascular and cellular ageing in patients with type 2 diabetes mellitus
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Background and aims: It is known that glucose disturbances contribute to micro- and macrovascular complications and vascular aging. The length of telomere (TL) is considered as a biomarker for vascular and cellular aging. But the interrelation of vascular aging and cellular aging in type 2 diabetes mellitus (T2DM) and pathogenic mechanisms of this interrelation remains a challenge. The aim of our study was to determine mechanisms of TL shortening and vascular aging in patients with T2DM.

Materials and methods: The study group included 50 patients with T2DM in mean age 58.4±7.83 years and 156 healthy patients in mean age 57.04±7.7 years. TL and telomerase activity (TA) was assessed by quantitative polymerase chain reaction (PCR). Intima-media thickness (IMT) and plaque presence (PP) were determined by ultrasonography in both left and right carotid arteries. Arterial stiffness (AS) was appreciated by aortic pulse wave velocity (PWV) measuring by SphygmoCor (AtCor Medical). Endothelial dysfunction as assessed by flow-mediated endothelium-dependent dilation (FMV) in response to reactive hyperemia and endothelium-independent vasodilation in response to nitroglycerine (NDV). Oxidative stress was assessed by malondialdehyde measuring, inflammation was estimated by interleukin-6 (IL-6), C-reactive protein (CRP), fibrinogen measuring.

Results: We found in group with T2DM compared with the control group of healthy patients a greater telomere shortening (9.57 vs 9.75, $p=0.0051$) and TA reduction (0.33 vs 0.49, $p=0.0023$). Vascular aging are more pronounced in patients with T2DM, than without diabetes: PWV CPIIB 12.3 m/s vs 11.3 m/c ($p=0.0032$), IMT 0.93 mm vs 0.77 mm ($p<0.0001$), PP 2.02 vs 1.29 ($p=0.0026$). Patients with T2DM have worse endothelial function: FMV 9 vs 11 ($p=0.0146$), NDV 13 vs 16.5 ($p=0.0001$). With regard to lipid oxidation product and inflammatory markers we found significant differences: patients with T2DM have more increased malondialdehyde (0.08 vs 0.02, $p=0.044$), CRP (3.60 vs 2.35, $p<0.0001$), increased fibrinogen (0.30 vs 0.11, $p=0.0007$).

Conclusion: Vascular aging (AS, IMT increasing, subclinical atherosclerosis (PP), endothelial dysfunction) and cellular aging (TL, TA reduction) are more pronounced in patients with T2DM, than without diabetes. Patients with T2DM have more pronounced oxidative stress and chronic inflammation. Thus we can suppose that oxidative stress and chronic inflammation play principal role in TL shortening and vascular aging at macro, tissue and cell level.

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Arterial stiffness is not associated with skin microvascular function in individuals with or without type 2 diabetes: the Maastricht Study

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Background and aims: Increased arterial stiffness leads to an increased pulsatile pressure load, which may damage the microcirculation. Individuals with type 2 diabetes (T2DM) may be particularly prone to the detrimental effects of this increased pressure load, because T2DM is associated with increased microvascular perfusion, allowing pulsatile pressure to penetrate deeply into the microcirculation. The skin enables the direct measurement of microcirculatory function both at rest and during provocative stimuli. Furthermore, the cutaneous microcirculation is considered a representative vascular bed to examine generalized microvascular phenomena. The aim of the present study was therefore to evaluate, in a large population-based cohort, the association between arterial stiffness and skin microvascular function. We additionally investigated whether any such association was stronger in individuals with as compared to those without T2DM.

Materials and methods: We used cross-sectional data of The Maastricht Study (for the present analysis: $n=737$; age 59.7 years; 45.2% women; 28.8% T2DM (by design)). The Maastricht Study is a population-based cohort study that focuses on the pathophysiology of T2DM. Arterial stiffness was determined via carotid-femoral pulse wave velocity (cfPWV, tonometry). In addition, finger skin capillaroscopy was used to determine capillary density at baseline and during post-occlusive hyperaemic response and venous congestion. Laser Doppler flowmetry was used to assess skin microvascular flow-motion.

Results: After adjustment for age and sex, both in individuals with and without T2DM, cfPWV was not associated with baseline capillary density, hyperaemic capillary recruitment, or capillary density during venous congestion (Table, models 1). In addition, cfPWV was not associated with microvascular flowmotion (models 1). Further adjustment for potential confounders (models 2 and 3) did not materially change these results. There was no interaction with type 2 diabetes (P for interaction, all $>.13$).

Conclusion: In the present population-based cohort study, arterial stiffness was not associated with skin microvascular function. This suggests that increased arterial stiffness does not lead to generalized microvascular dysfunction, irrespective of the presence of T2DM.

Table. Association between arterial stiffness and measures of skin microvascular function in individuals with and without type 2 diabetes

	Baseline capillary density (capillaries/mm ²)	Recruitment during peak reactive hyperaemia (%) ^A	Recruitment during venous congestion (%) ^A	Total skin flowmotion energy (AU) ^B
Regression coefficient (95% confidence interval) for +1 SD cfPWV				
Individuals with type 2 diabetes				
1	0.79 (-1.84; 3.42)	0.003 (-1.53; 1.53)	0.14 (-1.45; 1.73)	-0.05 (-0.15; 0.04)
2	0.06 (-2.82; 2.94)	0.32 (-1.35; 1.99)	0.39 (-1.34; 2.13)	-0.05 (-0.15; 0.06)
3	0.12 (-2.74; 3.00)	0.38 (-1.29; 2.06)	0.42 (-1.33; 2.16)	-0.04 (-0.14; 0.07)
Individuals without type 2 diabetes				
1	-1.63 (-3.31; 0.06)	1.22 (-0.41; 2.84)	1.50 (-0.25; 3.25)	-0.01 (-0.07; 0.06)
2	-1.56 (-3.51; 0.39)	0.78 (-1.10; 2.66)	1.01 (-1.01; 3.03)	-0.02 (-0.10; 0.06)
3	-1.20 (-3.17; 0.77)	0.41 (-1.49; 2.31)	0.62 (-1.42; 2.67)	-0.01 (-0.09; 0.07)

Model 1: adjusted for age and sex; model 2: additionally adjusted for heart rate and mean arterial pressure; model 3: additionally adjusted for waist-to-hip ratio, smoking habits, fasting glucose, total / high density lipoprotein cholesterol ratio, triglycerides, prior cardiovascular disease and use of lipid-lowering and anti-hypertensive medication.

^A Results were qualitatively similar when absolute differences in number of capillaries (delta) were used instead of percentage recruitment.

^B Analyses with flowmotion energy as the outcome were additionally adjusted for skin temperature in all models. Abbreviations: AU: arbitrary units; SD: standard deviation; cfPWV: carotid-to-femoral pulse wave velocity

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OP 17 Intra- and inter-islet cell signalling

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LKB1 and AMPK regulate *Nptx2* expression and glutamate signalling in pancreatic beta cells

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Background and aims: Inactivation in beta cells of the tumour suppressor Liver kinase B1 (LKB1/STK11) or the downstream enzyme AMP-activated protein kinase (AMPK) exerts dramatic effects on beta cell growth and insulin secretion. Here, we used massive parallel sequencing (RNASeq), and subsequent functional analyses, to identify gene clusters which may mediate these effects.

Materials and methods: Mice null for LKB1 or both AMPK catalytic ($\alpha 1$, $\alpha 2$) subunits were generated by Ins1Cre-mediated (beta cell-selective) deletion of *lkb1* alleles. Islets were isolated from four 12 week old mice per genotype. After 24 h culture at 11 mM glucose RNA was extracted (RNAEasy) before deep sequencing (RNASeq) on a HiSeq 2000. Cytosolic free Ca^{2+} was measured using fura-2 using an Olympus IX81 microscope with micromanipulator-controlled data capture via an Andor Zyla CMOS camera. Immunohistochemical analysis of pancreatic slices was performed using a rabbit anti-Nptx2 antibody on a Zeiss-200M microscope (Zen software), and analysed using ImageJ. Insulin secretion was measured during static incubations (30 min) at 3 or 16 mM glucose using radioimmunoassay.

Results: Amongst the mRNAs most strongly up-regulated by LKB1 deletion was a cluster involved with glutamate signalling, including *Nptx2*, encoding neuronal pentraxin 2 (11.2-fold, E-value <0.001), and *dlgap2*, encoding discs, large (Drosophila) homolog-associated protein 2 (11.9-fold, E<0.001). A similar degree of up-regulation was observed after AMPK deletion (19.7-fold, E<0.01 and 4.7-fold, E<0.01, respectively). LKB1 deletion increased Nptx2 immunoreactivity in islets by 6.0-fold, p<0.01. At 3 mM glucose, cytosolic calcium increases in response to the glutamate receptor agonist kainate were significantly increased by LKB1 deletion (area under the curve, 2.1-fold, p<0.05, amplitude 43 %, p<0.01), whilst insulin secretion was increased 2.2-fold (p<0.05) at 3 but not 16 mM glucose.

Conclusion: LKB1 regulates glutamate receptor signalling in beta cells by a mechanism likely to involve the up-regulation of Nptx2, a secreted protein which binds α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and by *dlgap2*, which interacts with PSD-95 (encoded by DLG4) and N-methyl-D-aspartate NMDA receptors. The actions of LKB1 are likely to involve the nutrient-sensitive protein kinase AMPK, and may thus provide a novel mechanism by which glucose or other secretagogues regulate insulin secretion or beta cell survival.

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The non-canonical NF κ B pathway as a novel player in beta cell dysfunction in diabetes

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Background and aims: We previously demonstrated that mice deficient for TRAF2 (β TRAF2) in their beta cells showed exacerbated glucose intolerance and an impaired first phase insulin secretion in a diet-induced obesity model. β TRAF2 islets also displayed dysregulated signaling as defined by hyperactivation of the non-canonical NF κ B signaling pathway. TRAF2 is an adaptor protein that functions by recruiting the E3 ubiquitin ligases BIRC2 and

BIRC3 to the complex, which target the NF κ B inducing kinase (NIK) for degradation thereby directly controlling the non-canonical NF κ B pathway.

Materials and methods: To dissect out the function of this E3 ubiquitin ligase complex in islet biology in diabetes, we generated a beta cell specific BIRC2/3 knockout (β BIRC) mouse. Of note, a class of BIRC inhibitors, so-called Smac mimetics, are in Phase I and II clinical trials as anti-cancer drugs. We hypothesized that genetic (β BIRC mice) or drug-based (Smac mimetic) deletion of BIRC would impair beta cell function.

Results: β BIRC mice showed increased glucose intolerance on a high-fat diet (45 kcal % fat) thus phenocopying β TRAF2. The common molecular denominator that we found, was increased cell intrinsic NIK accumulation and processing of p100 to p52 in unstimulated β TRAF2 and β BIRC islets, indicating activation of the non-canonical NF κ B pathway. Thus, disruption of the TRAF/BIRC E3 ligase complex derails control of non-canonical NF κ B signaling and thereby causes beta cell dysfunction. Next, we synthesized and purified the monovalent preclinical Smac-mimetic MV1. Treatment of C57BL/6 islets with MV1 resulted in increased processing of p100 to p52 indicating activation of the non-canonical NF κ B pathway. We then transplanted a limited and defined number of vehicle-control and MV1-treated islets into syngeneic diabetic mice and performed an i.p.GTT at day 3 and 10 post transplant. Both control and MV1-treated islets established normoglycemia at postoperative day 1-2. At day 3, MV1-treated islets showed a severe decrease in glucose tolerance compared to vehicle-treated controls. However, at day 10 there was no difference to controls, indicating that the MV1 Smac mimetic causes a transient defect in beta cell function. This raises the question whether cancer patients receiving Smac mimetics should be monitored for changes in glucose tolerance. In all models, β TRAF2 mice, β BIRC mice and MV1-treated islets, the loss of beta cell function was independent of cell death, as they maintained their islet mass.

Conclusion: Mice lacking TRAF2 or BIRC2/3 in beta cells exhibit severe defects in glucose homeostasis and insulin secretion in a diet-induced obesity model. At the molecular level this E3 ubiquitin ligase complex reigns in NIK activation as a non-canonical NF κ B pathway trigger. Loss of this control circuit by either genetic deletion or treatment with an anti-cancer Smac mimetic drug precipitates a diabetes phenotype. These data define NIK activation and non-canonical NF κ B as novel players in islet dysfunction, and commend them as potential drug targets in diabetes.

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Stimulation of insulin secretion by GPR75: identification of signalling pathways in rodent and human beta cells

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Background and aims: GPR75 is an atypical chemokine receptor that is activated by the pro-inflammatory chemokine ligand 5 (CCL5). We have previously demonstrated that GPR75 mRNA is highly expressed by mouse and human islets and it localises to β -cells, whereas the conventional CCL5-activated chemokine receptors CCR1, 3 and 5 are expressed at very low levels in islets. We have also shown that CCL5 elevates $[Ca^{2+}]_i$ in β -cells and stimulates insulin secretion, but the downstream signalling cascades and involvement of GPR75 in the stimulatory effects of CCL5 are unknown. This study therefore determined the pathways downstream of CCL5 signalling in mouse and human islets and investigated whether GPR75 activation is required for CCL5-induced insulin secretion.

Materials and methods: Changes in $[Ca^{2+}]_i$ were measured by single cell microfluorimetry of Fura-2-loaded human islet cells, insulin secretion from isolated mouse and human islets was determined in static incubation experiments and quantified by radioimmunoassay, and GPR75 was down-regulated by transient transfection with GPR75 siRNAs.

Results: Blockade of L-type Ca^{2+} channels reduced CCL5-induced elevations in $[Ca^{2+}]_i$ in human islets (10nM CCL5: 90 \pm 15% of ATP response; +10 μ M nifedipine: 14 \pm 6%, n=7-17, P<0.05), and opening of K_{ATP} channels was associated with a loss in CCL5 induced insulin secretion from mouse islets (2mM glucose: 0.19 \pm 0.02ng/islet/h; +25nM CCL5: 0.31 \pm 0.04, P<0.05; +25nM CCL5 +250 μ M diazoxide: 0.18 \pm 0.02, P>0.2 vs 2mM glucose). Inhibition of phospholipase C in human islets also abolished the secretory response to CCL5 (20mM glucose: 0.65 \pm 0.07ng/islet/h; +10nM CCL5: 1.22 \pm 0.25, P<0.05; +10nM CCL5 +10 μ M U73122: 0.44 \pm 0.07, P>0.2 vs 20mM glucose), as did depletion of typical isoforms of protein kinase C (PKC) by 24 hour exposure to 4 β PMA (control: 196 \pm 26% of 20mM glucose response; PKC

depleted: $90 \pm 10\%$, $n=7-8$, $P<0.01$). Furthermore, inhibition of the calcium-calmodulin-dependent protein kinase CAMK II diminished CCL5-induced insulin secretion from human islets (20mM glucose: 0.57 ± 0.08 ng/islet/h; $+10$ nM CCL5: 1.17 ± 0.15 , $P<0.05$; $+10$ nM CCL5 $+10 \mu$ M KN62: 0.69 ± 0.08 , $P>0.2$ vs 20mM glucose). Exposure of β -cells to GPR75 siRNAs for 48 hours caused $39.6 \pm 1.4\%$ reduction in GPR75 mRNA expression and this was accompanied by decreased insulin secretion in response to CCL5 (non-coding RNAs: $166 \pm 30\%$ of 20mM glucose response; GPR75 siRNAs: $70 \pm 10\%$, $n=6$, $P<0.05$).

Conclusion: These data indicate that CCL5 activates β -cell GPR75 to stimulate insulin secretion from mouse and human islets, which is dependent on signal transduction cascades that include phospholipase C activation, calcium influx via L-type Ca^{2+} channels, and activation of the calcium-dependent protein kinases PKC and CaMK II. This novel role for CCL5 in improving β -cell function via GPR75 activation may provide a potential therapeutic target for improving insulin secretion in type 2 diabetes.

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Knockout of GPR55 impairs insulin secretion and reduces islet cell turnover

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Background and aims: GPR55 is a G-protein coupled receptor (GPCR) whose activity is regulated by a range of synthetic and endogenous cannabinoids, and by lipid-derived ligands. We have previously reported that GPR55 knockout (KO) mice are more susceptible to diet-induced obesity, and the aim of the current study was to investigate the effect of GPR55 deletion on islet function in vivo and in vitro following diet-induced obesity.

Materials and methods: GPR55 KO mice and age-matched WT mice were fed ad lib either standard chow (SC, fat:protein:carbohydrate: 14%:28%:58%) or a high fat diet (HFD, 55%:16%:29%) for 19 weeks. Glucose and insulin tolerance tests were performed following a single i.p. administration of glucose (2g/kg body weight) or insulin (0.75U/kg body weight), and tail vein blood glucose concentrations were determined using a glucose meter. Islets isolated from WT and KO mice were lysed with acidified ethanol and insulin content was measured by radioimmunoassay. Dynamic insulin secretion was quantified by radioimmunoassay following perfusion of isolated islets. Islet caspase-3/7 activities were quantified using a luminescent assay following exposure to a cytokine cocktail (1U/ μ l IFN γ , 1U/ μ l TNF α , 0.05U/ μ l IL-1 β). BrdU (1mg/ml) was delivered to mice in their drinking water for 7 days prior to sacrifice, and beta cell proliferation was determined by insulin and BrdU immunostaining.

Results: GPR55 KO mice fed a HFD for 19 weeks were glucose intolerant (glucose at $t=30$, KO on SC: 20.8 ± 1.3 mM; KO on HFD: 28.1 ± 1.1 , $n=6$ $P<0.01$) and insulin resistant (reduction in blood glucose following insulin injection, KO on SC: 1.1 ± 0.1 mM; KO on HFD: 0.3 ± 0.4 , $n=6$ $P<0.05$). Islets isolated from KO mice fed a HFD for 19 weeks showed a small reduction in basal insulin secretion at 2mM glucose (KO: 0.11 ± 0.01 pg/islet/min; WT: 0.15 ± 0.03 , $n=4$ $P<0.05$) and glucose-stimulated insulin secretion was also impaired (peak responses to 20mM glucose, KO: 4.1 ± 1.3 pg/islet/min; WT: 8.5 ± 0.9 ; $P<0.05$). Islets from KO mice after maintenance for 19 weeks on SC showed increased cytokine-induced apoptosis (caspase 3/7 activities; luminescence, KO: $41,692 \pm 939$; WT: $29,980 \pm 457$; $n=8$ $P<0.01$) as did islets from KO mice fed on a HFD (KO: $55,093 \pm 2,740$; WT: $38,599 \pm 564$; $n=8$ $P<0.01$). In addition, while there was a 3.2 ± 0.6 -fold increase in beta cell BrdU incorporation in pancreases of WT mice fed a HFD this compensatory increase in beta cell proliferation in response to HFD was attenuated in KO mice (1.2 ± 0.2 -fold increase in beta cell BrdU incorporation; $n=12$, $P<0.05$ vs WT). Consistent with the reduced proliferation and increased apoptosis, islets isolated from HFD-fed KO mice had lower insulin content (KO: 134.8 ± 2.3 ng/islet; WT: 166.3 ± 4.2 ; $n=8$ $P<0.001$).

Conclusion: GPR55 KO mice show impaired glucose handling under conditions of diet-induced increased metabolic demand, which is associated with reduced insulin secretory capacity, increased islet cell apoptosis and insufficient compensatory increases in beta cell proliferation. These observations support GPR55 playing an important role in regulating islet function that could make it a suitable target for treating type 2 diabetes.

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IDs are novel oxidative stress-responsive genes in beta cells that regulate redox status and survival through effects on mitochondria and the NFE2L2 pathway

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Background and aims: Oxidative stress is a central mechanism of beta-cell glucotoxicity, but the underlying signaling pathways are only partially understood. Inhibitor of Differentiation (ID) proteins are transcriptional regulators induced by hyperglycaemia in islets, but the mechanisms involved and their role in beta-cells are not clear. Here we investigated: 1) whether oxidative stress regulates ID expression in beta-cells, and 2) the role of ID expression in beta-cell pathophysiology under conditions of oxidative stress.

Materials and methods: Fixed pancreata and isolated islets from diabetic db/db mice and their normoglycemic db/+ littermates were used to verify the expression of IDs and antioxidant genes. Insulin-secreting MIN6 beta-cells and isolated islets from Id1 and Id3-KO mice were cultured for 0-48h in the presence or absence of H_2O_2 (100-300 μ M) or ribose (5-50 mM) to induce oxidative stress. RNA interference was used to silence the expression of Id1 and/or Id3 in MIN6 cells. mRNA and protein levels were measured by real-time RT-PCR, western blot and immunocytochemistry. H_2O_2 levels were assessed by DCFDA probe, mitochondrial morphology by Mitotracker probe, oxygen consumption by Clark electrode and apoptosis by DNA fragmentation ELISA.

Results: ID1-4 expression was upregulated in the islets of diabetic db/db mice with parallel changes in the expression of multiple antioxidant genes. In MIN6 cells, ribose and H_2O_2 treatment increased the mRNA levels of Id1-4 in a time- and concentration-dependent manner with parallel changes in the expression of antioxidant genes. Furthermore, immunostaining showed that ribose treatment increased ID1 and ID3 nuclear localisation. In ribose-treated cells, siRNA-mediated inhibition of Id1 and/or Id3 reduced the expression of multiple antioxidant genes, including heme oxygenase. Additive effects were observed when both isoforms were inhibited. Glutathione peroxidase activity was also reduced after Id1/3 knockdown. These effects were accompanied by ~ 2 -fold increase in H_2O_2 levels ($p<0.01$), 18% reduction in oxygen consumption ($p<0.01$) and ~ 2 -fold increase in beta-cell apoptosis ($p<0.001$). Furthermore, Id1/3 inhibition induced mitochondrial fragmentation similar to that observed in the presence of ribose. Similarly, ribose-induced apoptosis in islets was potentiated in Id1-KO islets and to a stronger extent in Id3-KO islets (1.6-fold; $p<0.01$). Finally, under oxidative stress, Id1/3 inhibition further increased NFE2L2 nuclear localization but represses the expression of its interacting partners MafK and MafF.

Conclusion: We have identified IDs as a novel family of oxidative stress-responsive genes in beta-cells as well as an unexpected role for Id1 and Id3 in the modulation of redox status. Such modulation is likely to stem from the maintenance of an adequate mitochondrial adaptation and upholding of the expression of NFE2L2 interacting partners. The maintenance of an adequate mitochondrial-antioxidant response by IDs may promote beta-cell survival under conditions of oxidative stress.

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Optical interrogation of glucose-regulated beta cell connectivity

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Background and aims: The multicellular organization of beta cell dynamics produces a gain-of-function in insulin release through the generation of rhythmic and synchronous activity patterns. Here, we describe an optical silencing strategy to allow the precise and specific interrogation of the functional wiring patterns which orchestrate and pace beta cell interactivity.

Materials and methods: Functional multicellular calcium (Ca^{2+}) imaging (fMCI) was used in combination with online Monte Carlo-based correlation analyses to construct the wiring patterns underlying cell-cell connectivity and hormone release. Rapid Nipkow disk microscopy allowed video rate recording at speeds in excess of 5 Hz. Beta cell-specific expression of the light-activated inward chloride (Cl^-) channel, halorhopsin, was directed by crossing the Ins1Cre deleter strain with animals engineered to express

eNpHR3.0-EYFP following excision of a *loxP*-flanked STOP cassette. To allow user-directed single cell silencing within the field of view, a diffraction-limited 585nm laser was coupled via a fibre optic to a custom-manufactured dichroic array.

Results: Beta cells form a scale-free network which supports the synchronous propagation of glucose-stimulated Ca^{2+} waves by efficiently connecting distant regions of the intact islet ($n = 12$ recordings from 5 animals) (power law fit; $R^2 = 0.7247$). A typical feature of such a topology was the non-random appearance of superconnected hub cells whose firing activity repetitively preceded that of the remainder of the population. Online mapping of islet functional architecture using MATLAB routines coupled directly to the imaging setup revealed the presence of a statistically-stable hub cell population. Silencing of individual identified hubs using a pinpointing laser had catastrophic consequences for coordinated islet responses to glucose, and this could be reversed simply by ceasing illumination (11.5 ± 1.8 versus 3.1 ± 0.8 % correlated cell pairs, laser OFF versus laser ON, respectively; $P < 0.01$) ($n = 8$ recordings from 4 animals). By contrast, specific silencing of non-hub or follower cells was unable to significantly perturb islet dynamics (7.3 ± 1.8 versus 9.9 ± 2.0 % correlated cell pairs, laser OFF versus laser ON, respectively; $P > 0.05$) ($n = 7$ recordings from 4 animals). Further supporting a role for distinct wiring patterns in glucose-regulated islet connectivity, low-grade cytokine (IL-1 β and IL-6) treatment resulted in a dramatic and rapid collapse in correlated cell-cell activity due to impaired hub function (9.6 ± 0.9 versus 5.1 ± 0.6 % correlated cell pairs, 0 versus 4 hrs cytokine exposure, respectively; $P < 0.01$) ($n = 7$ recordings from 3 animals).

Conclusion: The intra-islet circuitry is dominated by superconnected hub cells which dictate population responses to glucose. Notably, these hubs are vulnerable to pro-inflammatory T2D insults and may contribute to the reduced functional beta cell mass that accompanies glucose intolerance.

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OP 18 Novel genes for type 2 diabetes and its complications

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A common Greenlandic *TBC1D4* variant confers muscle insulin resistance and type 2 diabetes

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Background and aims: Previous studies have shown that founder populations can be valuable for genetic association studies, because they have increased levels of linkage disequilibrium and may harbor deleterious mutations of higher frequency. The same can be argued for historically small and isolated populations. Motivated by this, and by the dramatically increased prevalence of type 2 diabetes in the small Greenlandic population, we performed association mapping of four type 2 diabetes-related quantitative traits, plasma glucose and serum insulin levels at fasting and at two hours during an OGTT, in Greenlandic individuals without known diabetes.

Materials and methods: Discovery analyses were performed in up to 2,757 participants of the Inuit Health in Transition cohort and replication in up to 1,064 participants from the B99 cohort. Participants underwent an OGTT. Samples were genotyped by the Illumina CardioMetabochip. Association testing was done using a linear mixed model in GEMMA to control for admixture and relatedness. Exome sequencing of selected individuals was done by Agilent SureSelect capture and Illumina sequencing.

Results: Applying array-based genotyping and exome sequencing, we discovered a nonsense variant in *TBC1D4* with an allele frequency of 17% in the Greenlandic population. Under a recessive model, this variant strongly increases the levels of plasma glucose ($\beta = 3.8$ mmol/L, $P = 2.5 \times 10^{-35}$) and serum insulin ($\beta = 165$ pmol/L, $P = 1.5 \times 10^{-20}$) two hours after an oral glucose load, while reducing fasting plasma glucose ($\beta = -0.18$ mmol/L, $P = 1.1 \times 10^{-6}$) and fasting serum insulin (-8.3 pmol/L, $P = 0.0014$). Even the heterozygous carriers have an increased 2 hour plasma glucose ($\beta = 0.43$ mmol/L, $P = 5.3 \times 10^{-5}$). The same polymorphism was associated with an increased risk of type 2 diabetes risk (OR 10.3, $P = 1.6 \times 10^{-24}$). All findings were replicated in the B99 cohort. Analyses of muscle biopsies from mutation carriers showed decreased mRNA and protein expression of the long muscle-specific isoform of *TBC1D4* and decreased muscle protein abundance of GLUT4 projecting a decreased insulin-stimulated glucose uptake in skeletal muscle.

Conclusion: We establish a single variant which imposes effect sizes several times larger than any findings of large-scale genome-wide association studies and accounts for more than 10% of all cases of type 2 diabetes in a population. This finding constitutes further proof that it is valuable to conduct genetic association studies outside the traditional setting of large homogeneous populations.

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Fine-mapping type 2 diabetes susceptibility loci with high-density imputation

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Background and aims: Genome-wide association studies (GWAS) have been successful in identifying loci for type 2 diabetes (T2D). These loci are typi-

cally characterised by common lead SNPs with association signals that extend over large genomic intervals. Consequently, there has been limited progress in localising causal variants for T2D and establishing the functional impact of these loci in the pathogenesis of the disease. We aimed to: (i) identify multiple signals of association with T2D in established loci; (ii) assess the evidence for association with low-frequency (LF) variants, minor allele frequency (MAF) <5%, in established loci; and (iii) localise the likely causal variant(s) for each association signal.

Materials and methods: We combined Metabochip and GWAS data from 46,168 T2D cases and 172,714 controls of European ancestry, supplemented by imputation up to the 1000 Genomes Project reference panel (March 2012 release), to facilitate fine-mapping of 39 established loci. We undertook approximate conditional analyses implemented in GCTA, using 3,298 cases and 3,708 controls from GoDARTS as reference, to identify loci with multiple signals of association with T2D at “locus-wide” significance ($p < 10^{-5}$). We defined “credible sets” of SNPs that account for 99% of the probability of including the causal variant for each association signal.

Results: We identified five association signals mapping to the *KCNQ1* locus, three at *HNF1A*, and two each at *CDKN2A/B*, *DGKB*, *MC4R*, *GIPR* and *HNF4A*. We highlighted LF associated variants mapping near *HNF1A* (rs1800574, MAF=2.2%, $p=4.2 \times 10^{-10}$), *HNF4A* (rs1800961, MAF=3.9%, $p=1.4 \times 10^{-9}$), and *MC4R* (rs17066842, MAF=4.8%, $p=2.9 \times 10^{-6}$), all independent ($r^2 < 0.05$) of the common lead GWAS SNPs at these loci. The 99% credible set includes no more than ten variants at nine loci, with greatest refinement at *MTNR1B* (rs10830963 only), one signal at *KCNQ1* (3 SNPs map to 197bp), and *TCF7L2* (3 SNPs map to 4.3kb), and with both signals at *CDKN2A/B* each mapping to less than 1.5kb. The variants in the 99% credible sets at these nine “fine-mapped” loci are common (MAF $\geq 5\%$) and predominantly non-coding, with the exception of rs13266634 (R325W) at *SLC30A8*.

Conclusion: Our study has provided insight into the genetic architecture of T2D and has prioritised regions for follow-up with regulatory annotation that may aid characterisation of the functional role of these GWAS loci in the pathogenesis of the disease.

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Identification of protein-coding variants associated with risk of type 2 diabetes

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Background and aims: Genome wide association studies (GWAS) have successfully identified common [minor allele frequency (MAF) >5%] genetic variants contributing to risk of type 2 diabetes (T2D). However, the impact of low-frequency (LF; MAF 0.5–5%) and rare (MAF <0.5%) variants to disease susceptibility remains widely unexplored. We evaluated the role of this class of variation to T2D predisposition, focusing on coding variants as these are enriched for functional impact and allow more direct insight into disease biology.

Materials and methods: We analysed: (i) exome sequence data from 12,940 individuals (6,504 T2D cases, 6,436 controls), ascertained from five major ethnic groups (European, South Asian, East Asian, Hispanic and African American); and (ii) exome-array data from additional 81,740 Europeans (29,569 T2D cases, 52,171 controls). We first performed single-variant analyses within each ethnic group, and then combined ancestry-specific association summary statistics in a trans-ethnic meta-analysis using MANTRA. We defined exome-wide significance by a \log_{10} Bayes' Factors (BF) >5. Conditional analysis was done to determine if coding variants drive the known common SNP GWAS signal, or represent statistically independent associations.

Results: In the combined analysis, 26 coding variants, mapping to 18 loci, were associated with T2D at exome-wide significance. All except two have MAF >5% in the ethnic group driving the association signal. Of these, seven variants at five loci (*SLC30A8*, *MACF1*, *GCKR*, *PPARG*, and *KCNJ11-ABCC8*) were confirmatory of previously-reported common coding variants driving GWAS signals. Of the remaining 19 T2D-associated coding variants, 13 also mapped to established GWAS loci but have not previously been reported to be causal. These included *PAX4* R192H (rs2233580, \log_{10} BF >6.3), a signal driven exclusively by East Asians (odds ratio=1.79[1.59–1.99], MAF ~10%). Reciprocal conditional analyses at these loci revealed the novel coding variants to be either independent of, or indistinguishable from previously report-

ed lead GWAS SNPs, and implicate several novel genes - including *RREB1*, *THADA*, and *TM6SF2* - in T2D pathogenesis. The remaining six variants, mapping to three loci, were located entirely outside known GWAS regions. These include two variants in *PAM* (D563G, \log_{10} BF=9.96) and *PP1P5K2* (S1207G, \log_{10} BF=7.47), for which a T2D-association was recently described in the Icelandic population. Three further highly-correlated coding variants at *MTMR3/ASCC2* were associated with T2D (Europeans specific signal). The *MTMR3* variant (\log_{10} BF=6.46) has the strongest residual association signal after accounting for the other variants and represents the most plausible candidate at this novel locus. The last novel locus was *FAM63A* (\log_{10} BF=5.24) and signal was driven by a LF variant. The T2D risk-allele has a MAF of 1% in Europeans, and was otherwise only detected in South Asian sequence data (11 copies, $p=0.02$ for association).

Conclusion: Here we show that rare and LF coding variants of moderate to large effect are not major contributors to T2D risk. We identify 26 coding variants with exome-wide significant associations with T2D and highlight the promise of this approach to provide insight into the pathophysiology of the disease.

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Meta-analysis of birth weight genome-wide association studies identifies two novel loci extending links between early growth and adult metabolic diseases

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Background and aims: Lower birth weight (BW) is associated with increased risk of future type 2 diabetes (T2D) and cardiovascular diseases. Based on HapMap 2 imputation, we previously reported 7 loci associated with BW, of which two (*ADCY5* and *CDKALI*) have been implicated in T2D and one (*ADRB1*) in hypertension. We have expanded this effort by increasing sample size and undertaking imputation up to 20.8M SNPs from the more dense 1000 Genomes Project reference panels. With these data we aimed to 1) discover novel loci; 2) assess evidence of association with low-frequency variants (minor allele frequency <5%) of larger effect sizes; and 3) fine-map established and novel loci by constructing credible sets of variants that are most likely to be causal on the basis of statistical evidence of association.

Materials and methods: For analysis, we considered 41,551 European singletons born at ≥ 37 weeks' gestation with genome-wide association (GWA) and imputation data from 17 studies. Standardized sex-specific Z-scores of BW were tested for association with each SNP assuming an additive genetic model after adjustment for gestational week where available. Association summary statistics were combined across studies using inverse-variance fixed-effects meta-analysis.

Results: Two novel common variant loci were detected at genome-wide significance: near *MAFB* ($p=3.1 \times 10^{-8}$) and at *SREBF2* ($p=3.9 \times 10^{-9}$). *MAFB* has been implicated in hyperlipidemia and *SREBF2* is involved in cholesterol biosynthesis. There was no evidence for low-frequency variants that explain common GWA signals. The 99% credible sets included fewer than 20 SNPs at 4 loci. At *ADRB1*, the credible set included just 5 variants, including G389R. We will further refine the association signals by combining with GWA studies of non-European ancestry.

Conclusion: Collectively, 4 of the 9 known and novel loci provide genetic links between BW and adult metabolic phenotypes such as T2D, hypertension and hyperlipidemia, highlighting complex non-linear relationships between genetic variation, early growth and later metabolic disease including T2D.

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Functional SNPs as focused gene probes: results from SUMMIT on diabetic nephropathy, coronary and lower extremity artery disease

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Background and aims: Diabetic nephropathy (DN), coronary (CAD) and lower extremity artery disease (LEAD) are devastating, but, at least partially, heritable complications of diabetes with a poorly understood mechanistic basis. To move towards more effective preventative and therapeutic strategies, the SUMMIT consortium has conducted large-scale genome-wide association studies. Despite large sample sizes for CAD (4009 cases and 7918 controls), LEAD (2345 cases and 8706 controls), and specific sub-phenotype interrogation in DN (5908 cases and 4965 controls), only 3 genome wide significant signals ($p < 5 \times 10^{-8}$) were detected. This might imply a highly polygenic architecture of the considered phenotypes or significant noise levels in the trait definitions. At the same time, repositories such as NHGRI's GWAS catalog contain thousands of validated loci associated with a variety of traits. Each of these is likely tagging a functional alteration influencing a specific gene product. Focusing on these prior associations and their implicated genes can eliminate millions of non-functional SNPs to pinpoint variants associated with diabetic complications.

Materials and methods: We acquired all SNPs associated with any trait from NHGRI's GWAS catalog as well as cis-expression QTLs from the GTEx project. SNPs were pruned at a significance level of $p < 1 \times 10^{-5}$ and reduced to lead SNPs using a p-value-dependent distance cutoff. We mapped SNPs to probable causal genes using a novel LD and distance-based algorithm that has been validated to provide an F-measure of 73% on metabolite QTLs. This resulted in a total of 11,000 SNPs associated with over 2,000 phenotypes which map to 6,500 genes. As a measure of overall signal improvement, we compared QQ-plots before and after limiting analysis to gene probe SNPs based on a Wilcoxon rank-sum test of the truncated log p-values. Finally, we computed false discovery rate estimates for all SNPs in the full and the restricted set and report variants passing an FDR cutoff of 0.3 as candidates for specific signals discovered through the method.

Results: We obtained significant improvements for the QQ-plots on CAD, LEAD (with various stratifications) and eGFR traits (all p-values < 0.01). For instance, rs7173743 in the *MORF4L1* gene is associated with CAD on the background of type 2 diabetes at a q-value of 0.07 in the focused set whereas it was insignificant (q-value = 1) in the full set. When combining SUMMIT CAD data with CARDIOGRAM data, this locus reaches genome-wide significance. Other examples include rs13180 in the *IREB2* gene for LEAD in smokers (q-value < 0.01) and rs13333226 in the *UMOD* gene (q-value < 0.08) for eGFR.

Conclusion: Overall we can implicate a number of plausible loci stemming from robust prior phenotype associations in traits studied by SUMMIT on the background of diabetes. We plan to explore these results on the basis of their associated genes in pathway-based analysis to derive mechanistic insights for the traits under consideration in the SUMMIT consortium. This approach appears useful for finding novel associations for previously identified loci when the full GWAS lacks power to identify new loci achieving traditional genome-wide significance.

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Genome-wide association study for diabetic retinopathy in Japanese patients with type 2 diabetes

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Background and aims: Diabetic retinopathy (DR) is one of the leading causes of blindness in Japanese adults. Several reports have shown familial aggregations of DR or advanced DR among patients with type 1 or type 2 dia-

betes, suggesting genetic susceptibility contributes to the development and/or progression of DR. Although several candidate-genes have been shown to be associated with susceptibility to DR, namely genes encoding Angiotensin-I Converting Enzyme (*ACE*), Aldose reductase (*AKR1B1*), Vascular Endothelial Growth factor A (*VEGFA*) and Erythropoietin (*EPO*), the association of these genes with DR has not reached to genome-wide significance levels (p -value $< 5.0 \times 10^{-8}$) except rs1617640 in the *EPO* locus. Genome-wide association studies (GWAS) for advanced DR in European population have identified several suggestive loci with borderline association (p -value $< 1 \times 10^{-6}$), however the association of these loci with DR is still inconclusive. To identify novel genetic loci associated with the susceptibility to DR, we performed GWAS for DR in Japanese patients with type 2 diabetes.

Materials and methods: We divided Japanese patients with type 2 diabetes, registered in BioBank Japan, into 2 groups; 1) DR cases, defined as patients having any stages of DR, and 2) controls, who did not have any sign of DR and with long duration of diabetes (≥ 10 years) or with diabetic nephropathy. We examined 2 independent case-control groups (Study-1; 4,839 DR cases and 4,041 controls, Study-2; 693 DR cases and 1,524 controls) for ~ 7.5 million single nucleotide polymorphisms (SNPs) from directly genotyped data (Study-1; Omni-express, Study-2; Illumina 610K) and genotype imputation using mini-MACH. Results of the 2 GWAS were combined with an inverse variance method in a fixed effect model.

Results: We identified 5 SNP loci associated with DR in Japanese patients with type 2 diabetes (rs8025089 on chromosome [Ch]15: $p = 3.0 \times 10^{-7}$, Odds ratio [OR] = 1.15, 95% confidence interval [CI], 1.09-1.22, rs12630354 on Ch3: $p = 4.2 \times 10^{-7}$, OR = 1.15, 95% CI, 1.09-1.22, rs2471299 on Ch7: $p = 1.5 \times 10^{-6}$, OR = 1.14, 95% CI, 1.08-1.20, rs2517532 on Ch6: $p = 3.9 \times 10^{-6}$, OR = 1.13, 95% CI, 1.08-1.19, rs4240499 on Ch10: $p = 4.4 \times 10^{-6}$, OR = 1.17, 95% CI, 1.10-1.26). A sub-group analysis, excluding patients with simple DR, has identified 3 loci associated with advanced DR (2,003 DR cases [Study-1] and 368 DR cases [Study-2]) ($p < 1 \times 10^{-3}$). None of the association reached to genome-wide significance level ($p < 5.0 \times 10^{-8}$) in this study. Regarding previously reported DR susceptible loci (*ACE*, *AKR1B1*, *VEGFA* and *EPO*), none of these loci was significantly associated with DR in the present study.

Conclusion: We have identified several candidate loci potentially contributing to DR susceptibility, although further studies are required to validate the association of these loci with DR.

OP 19 Insulin: clinical decision making

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Clinical factors influencing the basal rate profile in subjects with type 1 diabetes treated with continuous subcutaneous insulin infusion (insulin pumps)

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Background and aims: Defining an individually appropriate basal rate profile in patients with type 1 diabetes requires knowledge about the general shape of such a profile, and needs to take into account patient factors with a significant influence on (a) the total dose of insulin administered as a basal rate and (b) the circadian variation in insulin needs. We assessed these influences based on a retrospective analysis of 339 patients, in whom the adequacy of basal rates was checked by means of a 24 h fasting period.

Materials and methods: Laboratory-based blood glucose profiles determined during regular day and night (eating meals) and during a 24 h fasting period (6 p.m. to 6 p.m.) and basal rate profiles corrected after the fasting period were entered into our database for 339 patients with type 1 diabetes during inpatient treatment in a specialized diabetes centre (183 women, 156 men; age 41 ± 14 years, HbA_{1c} 8.3 ± 1.6 %; BMI 26.1 ± 4.9 kg/m²; diabetes duration 20 ± 12 years; insulin dose 0.55 ± 0.20 IU/kg body weight per day). Patients were divided according to gender, or into tertiles of age, body-mass-index, and diabetes duration. A significant influence of the characteristic in question was assessed by repeated-measures ANOVA on hourly basal rates and also expressed as a percentage of the overall 24 h basal rate for each individual hour.

Results: A 24 h fasting period resulted in relatively stable blood glucose values of 6.6 ± 2.8 mmol/L with 11.5 % of the values > 9.9 mmol/L and 11.3 % of the values < 3.7 mmol/L. Basal rates after correction for low or high blood glucose values during the fasting period showed typical circadian rhythms with “dawn-” and “dusk-” phenomena and a total (mean \pm SD) basal rate of 21.5 ± 9.2 IU/day. There were significant differences due to gender (more prominent dawn phenomenon in males; $p < 0.0001$), age (more prominent dawn phenomenon in younger subjects; $p = 0.036$), body mass index (higher overall basal rates in more obese subjects; $p < 0.0001$; with no influence on the circadian distribution $p = 1.00$), and diabetes duration (more prominent dawn phenomenon in those with shorter diabetes duration; $p < 0.0001$).

Conclusion: Our large database helps define basal rate profiles for subjects with type 1 diabetes treated with continuous subcutaneous insulin infusions. It also allows to take into account clinical characteristics of the patients with a significant influence on the basal rate profile (both total amount of insulin and circadian profile) to individually predict insulin requirements during insulin pump treatment. Based on multivariate regression analysis, it may be possible to predict individual hourly basal rates with reasonable confidence intervals.

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Relationship of HbA_{1c} with body weight change over 4 years from starting insulin therapy in people with type 2 diabetes: the CREDIT study

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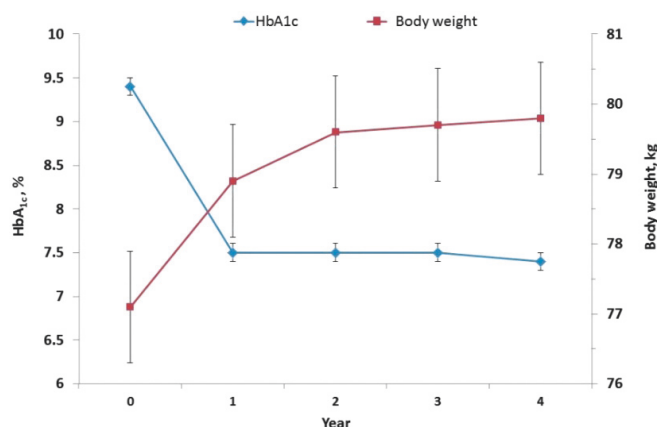
Background and aims: Insulin treatment is often delayed for people with type 2 diabetes; fear of weight gain and any consequences of glycaemic control can factor in that delay. Clinical trials provide some insight into this issue but have limited generalisability due to restrictions on participant selection and treatments used. Here, we examine the relationship between change in body weight and HbA_{1c} over 4 years of insulin therapy in routine clinical practice.

Materials and methods: CREDIT was a noninterventional study of people with type 2 diabetes beginning insulin therapy in Europe, North America, and Asia. Physicians were free to choose and adapt treatments in accordance with usual practice; data were collected from physician records. Univariate

and multivariable analyses were used to determine the relationship between change in body weight and HbA_{1c} over 4 years.

Results: Determinants of HbA_{1c} were studied in 1973 people with 4-year data from starting insulin; 47% were men, baseline mean age 61 ± 10 (SD) years, BMI 29.2 ± 6.1 kg/m², 10 ± 8 years of diabetes, HbA_{1c} 9.5 ± 1.9 % (80 ± 21 mmol/mol), 69% continuing oral glucose-lowering therapies. Half were started on basal insulin alone, 23% on premix insulin alone, and 15% on basal + meal-time insulin; over all insulin regimens, starting insulin dose was 0.26 ± 0.18 U/kg. In the first year, HbA_{1c} declined, then remained stable (Figure), with a decrease at year 4 of -2.0 ± 2.1 % (-22 ± 23 mmol/mol). Body weight increased an average of 1.9 ± 4.7 kg in the first year, but < 1.0 kg over the subsequent 3 years; over the 4 years, weight increased by 2.6 ± 7.4 kg. Daily insulin dose increased 0.15 ± 0.23 U/kg over the first year and by 0.27 ± 0.31 U/kg over the 4 years. This was accompanied by a change in insulin regimen from starting insulin to 4 years for 44% of population (30% on basal alone, 33% on basal + meal-time, and 25% on premix alone at year 4). After adjusting for region and centre, 4-year change in weight was predictive of HbA_{1c} at year 4 ($P < 0.0001$), but with a small effect size: a weight gain of 2.6 kg was associated with a 0.005 %-unit (0.05 mmol/mol) higher HbA_{1c} at year 4. Other variables were associated with HbA_{1c} at year 4: baseline HbA_{1c} , BMI, age, and at 4 years, insulin dose, number of oral glucose-lowering drugs and symptomatic hypoglycaemia. Taking these variables into account, weight change remained predictive of HbA_{1c} at year 4 ($P < 0.04$). Symmetrically, the odds ratio (95% CI) for an HbA_{1c} below 7.0 % vs. $HbA_{1c} \geq 7.0$ % at year 4 following a 1.0 kg increase in weight over the 4-year period, was 0.97 (0.96, 0.99; $P = 0.0015$), after adjusting for other predictive variables.

Conclusion: After 4 years of insulin treatment in people with type 2 diabetes, weight gain was associated with minimally higher HbA_{1c} values at year 4. These results should allay fears of weight gain having a strong impact on glycaemic control.



Supported by: Sanofi

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The INITIATOR study: real-world treatment patterns and outcomes in patients with type 2 diabetes initiating insulin glargine or liraglutide

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Background and aims: Patients with type 2 diabetes mellitus (T2DM) not achieving glycaemic control on oral antidiabetic drugs (OADs) can initiate injectable therapy with insulin or a glucagon-like peptide-1 receptor agonist (GLP-1) analogue. As the first large real-world observational longitudinal study of T2DM patients Initiating New Injectable Treatment Introduced after Anti-diabetic Therapy with Oral-only Regimens (INITIATOR), this analysis aimed to provide a comprehensive understanding of patient characteristics, treatment patterns, and associated outcomes.

Materials and methods: T2DM patients from 2 of the largest US commercial health insurers who were aged ≥ 18 years, previously on OADs only, and initiated either insulin glargine pen (GLA) or the GLP1 analogue liraglutide (LIRA) between April 1, 2010–March 31, 2012 were included. Patients had continuous healthcare coverage during the 6 months before (baseline [BL]),

and 12 months after initiation (follow-up). Health claims and medical chart data from eligible patients were extracted by OP and HC from their respective health plans. We compared differences in BL characteristics between treatment groups and 1-year follow-up measures to BL within each group. Treatment patterns, and clinical and economic outcomes at follow-up were assessed descriptively within each health plan.

Results: 4,490 patients were included (45.5% women; mean age 52.7 years; mean number of OADs 2). Significant BL differences existed: GLA patients had higher comorbidity burden, higher HbA_{1c}, lower weight, and were less often women (Table). A total of 23.8% of LIRA patients had a HbA_{1c} level of < 7.0% and 21.4% reported 'improve weight control' as a reason for initiation. At 1 year follow-up, overall treatment persistence was 64.0% for GLA and 49.4% for LIRA patients; 3.5% of GLA patients were on twice-daily GLA. Both groups had significant HbA_{1c} reductions. GLA patients had a slight weight gain whereas LIRA patients lost weight. Overall follow-up hypoglycaemia rates were 16% in GLA and 9% in LIRA patients; rates of severe hypoglycaemia were low. Compared with BL, LIRA patients had significant increases in total healthcare costs (OP and HC), in contrast to GLA patients who had fewer diabetes-related hospitalizations (OP only) and no increase in total healthcare costs. LIRA (OP and HC) and GLA patients (OP only) incurred higher diabetes drug costs.

Conclusion: These findings showed clinically relevant differences in BL characteristics and follow-up outcomes in T2DM patients initiating GLA or LIRA. This study highlights challenges in translating clinical trial findings into real-world settings, and in conducting comparative effectiveness studies where important BL group differences may exist.

Table. INITIATOR Study: Patient Characteristics and Outcomes.

	OP ^a GLA (n = 1,278)	OP ^a LIRA (n = 1,469)	HC ^b GLA (n = 838)	HC ^b LIRA (n = 905)
BL age, mean (SD) ^c , years	53.3 (8.8)	52.2 (8.9)**	53.2 (9.0)	52.4 (8.6)
Female sex, n (%)	553 (43.3)	714 (48.6)*	341 (40.7)	434 (48.0)*
BL weight, mean (SD) ^c , kg	100.8 (23.3)	111.1 (24.7)**	101.2 (23.6)	110.6 (23.7)**
BL HbA _{1c} , mean (SD) ^c , %	9.73 (2.08)	8.23 (1.76)**	9.72 (2.07)	8.12 (1.55)**
BL Quan-Charlson Comorbidity Score, mean (SD) ^c	0.89 (1.53)	0.61 (1.14)**	0.87 (1.53)	0.65 (1.21)**
Follow-up persistence ^d , n (%)	802 (62.8)	735 (50.0)	553 (66.0)	437 (48.3)
Follow-up HbA _{1c} ^e , mean change (SD), %	-1.24 (2.26)	-0.53 (1.59)	-1.23 (2.09)	-0.48 (1.65)
Follow-up weight ^f , mean change (SD), kg	1.14 (6.14)	-2.57 (7.15)	1.20 (8.57)	-2.99 (6.44)
Follow-up hypoglycaemia ^g /severe hypoglycaemia ^h , n (%)	210 (16.4)/18 (1.4)	128 (8.71)/6 (0.4)	134 (16.0)/17 (2.0)	95 (10.5)/5 (0.6)
Diabetes-related hospitalization, n (%)	BL: 115 (9.0) HY2: 65 (5.1)**	BL: 39 (2.7) HY2: 56 (3.8)	BL: 52 (6.2) HY2: 57 (6.8)	BL: 40 (4.4) HY2: 46 (5.1)
Diabetes-related healthcare cost, mean (SD) ⁱ	BL: \$3,301 (\$8,623) HY2: \$3,474 (\$10,410)	BL: \$1,947 (\$4,538) HY2: \$3,026 (\$6,145)**	BL: \$3,783 (\$19,359) HY2: \$3,665 (\$16,474)	BL: \$2,320 (\$4,157) HY2: \$3,636 (\$8,192)**
Diabetes drug costs, mean (SD) ^j	BL: \$641 (\$703) HY2: \$1,199 (\$1,013)**	BL: \$661 (\$708) HY2: \$1,520 (\$1,025)**	BL: \$739 (\$806) HY2: \$611 (\$830)**	BL: \$764 (\$793) HY2: \$1,587 (\$1,164)**

*p < 0.05; **p < 0.001. ^aData collected from two health insurance plans: with Optum™ (OP) and HealthCore™ (HC). ^bStatistical significance denotes differences between treatment cohorts within each health plan. ^cPersistence to index medication. ^dAmong patients with ≥ 1 HbA_{1c} result or ≥ 1 weight reading in the follow-up period. ^eBased on hypoglycaemia events recorded in patient charts. ^fSevere hypoglycaemia is defined as events requiring inpatient or emergency room treatment based on medical claims with hypoglycaemia diagnosis. ^gHospitalizations and costs assessed during the BL period and during the second 6 months of follow-up (HY2); statistical significance denotes change from BL to follow-up within each treatment arm.

Supported by: Sanofi US, Inc.

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Characteristics of patients with type 2 diabetes mellitus (T2DM) on basal insulin who do not achieve glycaemic goals

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Background and aims: Despite the efficacy of basal insulin therapy in individuals with T2DM, a significant number of patients may not achieve glycaemic goals. Patients taking basal insulin who reach their fasting plasma glucose (FPG) goal but not their glycated haemoglobin A_{1c} (HbA_{1c}) target represent an important unmet T2DM management need. This study used both randomized clinical trial (RCT) and "real-world" data to compare the baseline characteristics of T2DM patients with HbA_{1c} ≥ 7.0% achieving FPG levels < and ≥ 130 mg/dL.

Materials and methods: Characteristics, including age, gender, BMI, and weight, of patients on basal insulin who do or do not achieve HbA_{1c} < 7.0% were analysed using 11 pooled RCTs assessing insulin glargine (6-months follow-up) and real-world data from the GE Centricity electronic medical records (EMR) database (6- and 12-months follow-up). Patients with HbA_{1c} ≥ 7.0% were stratified by FPG level (< 130 or ≥ 130 mg/dL).

Results: Patients with high HbA_{1c} at follow-up also had high baseline HbA_{1c} (HbA_{1c} ≥ 7.0% vs < 7.0%: RCT, 9.1% vs 8.5%; EMR: 9.0% vs 8.1% [6-months

follow-up] and 9.0% vs 8.0% [12-months follow-up]). About 50% of the RCT patients reached HbA_{1c} goal vs < 30% of EMR patients. Of patients with HbA_{1c} ≥ 7.0%, about 50% (RCT) and < 30% (EMR) achieved FPG < 130 mg/dL. RCT patients tended to be younger, less obese, and weigh less vs EMR patients (mean: age 2-4 years younger; BMI ~3 kg/m² less; and weight ~6 kg less). Patients with HbA_{1c} < 7.0% were somewhat older than those with HbA_{1c} ≥ 7.0% (mean age in years for HbA_{1c} < 7.0% vs ≥ 7.0%: RCT, 58.4 vs 57.7; EMR, 62.3 vs 60.2 [6-months follow-up] and 62.7 vs 59.8 [12-months follow-up]). Little difference existed in gender distribution or mean BMI for patients with HbA_{1c} < 7.0% versus HbA_{1c} ≥ 7.0%. For both RCT and EMR patients with HbA_{1c} ≥ 7.0%, those with FPG < 130 mg/dL were slightly older than those with FPG ≥ 130 mg/dL (mean age for FPG < 130 vs ≥ 130 mg/dL: RCT, 58.7 vs 56.4 years; EMR, 62.3 vs 59.6 [6-months follow-up] and 62.4 vs 59.0 [12-months follow-up]). Differences were also seen in baseline insulin usage in the RCT patients, with more patients with FPG ≥ 130 mg/dL using a fast-acting insulin analogue (71.2% vs 28.9%). For both RCT and EMR patients, other demographic characteristics did not differ between the FPG groups.

Conclusion: We present differences in characteristics between patients on basal insulin who do or do not achieve HbA_{1c} < 7.0%. Patients with a higher baseline HbA_{1c} were less likely to achieve target HbA_{1c} < 7.0% on basal insulin alone; more intensive treatment regimens may be required. A large proportion of the HbA_{1c} > 7.0% patients were also above target for FPG at follow-up and would likely benefit from addition basal insulin titration. In patients achieving FPG < 130 mg/dL, despite above target HbA_{1c} values, postprandial glucose (PPG) control could be used to help bring their HbA_{1c} within recommended limits. Appropriate therapeutic choices for those not reaching HbA_{1c} < 7.0% should be based on assessment of FPG and PPG, as well as of HbA_{1c}. A better understanding of patient characteristics and factors involved in goal achievement might increase the numbers of patients reaching targets and reduce the time they are exposed to hyperglycaemia and its adverse consequences.

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People with type 2 diabetes with lower HbA_{1c} using insulin experience fewer cardiovascular events and death: results from the CREDIT study

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Background and aims: CREDIT (Cardiovascular Risk Evaluation in people with Type 2 Diabetes on Insulin Therapy) was a noninterventional study designed to examine relationships between HbA_{1c} and cardiovascular (CV) events in 2999 patients beginning insulin in real-world practice in Europe, North America, and Asia, with up to 54 months' follow-up. The study did not impact normal practice, having no intervention or formal visits-instead, data were collected from physician reports.

Materials and methods: Primary outcome was the composite of nonfatal stroke or myocardial infarction (MI) or CV death. Events were blindly adjudicated by an endpoints committee. Relative hazard of CV events was described with Cox proportional hazards models, including patient risk factors, and updated mean HbA_{1c} as a time-dependent covariate. Primary outcome components were described separately. The relationship of severe and symptomatic hypoglycaemia (collected observationally for prior 6 months) to CV and all-cause mortality was examined by adding patient-level covariates to the Cox models.

Results: In total, 147 primary events were accrued during study follow-up. There were 60 CV deaths, 44 nonfatal MIs, and 57 nonfatal strokes, with 148 all-cause deaths. There was a significant positive relationship between updated mean HbA_{1c} and primary outcome; hazard ratio (HR) 1.25 (95% CI: 1.12-1.40; P<0.0001). CV death (HR 1.31 [1.10-1.57]; P=0.0027) and stroke (HR 1.36 [1.17-1.59]; P<0.0001) were both strongly associated with HbA_{1c}, but MI was not (HR 1.05 [0.83-1.32]). One or more severe hypoglycaemic episodes affected 175 participants, while 1508 experienced ≥ 1 symptomatic hypoglycaemic event. We found no relationship between history of severe or symptomatic hypoglycaemic events and CV or all-cause death.

Conclusion: Thus, as measured in the CREDIT study, ongoing poorer glucose control was associated with CV events; hypoglycaemia was not associated with CV or all-cause death.

Supported by: Sanofi

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Intensive versus conservative glucose control in patients undergoing coronary artery bypass graft surgery (GLUCO-CABG Trial)D. Smiley¹, S. Cardona¹, F. Pasquel¹, F. Farrokh¹, S. Jacobs¹, L. Peng², M. Halkos¹, J.D. Puskas¹, R.A. Guyton¹, V. Thourani¹, G.E. Umpierrez¹;¹Medicine-Division of Endocrinology, Emory University,²Biostatistics, Rollins School of Public Health, Atlanta, USA.

Background and aims: This randomized controlled trial aimed to determine whether intensive glucose control (intensive, BG target: 5.6–7.8 mmol/L) reduces perioperative complications compared to conservative glucose control (conservative, BG target: 7.8–10 mmol/L) in hyperglycaemic patients undergoing CABG.

Materials and methods: After ICU care, subjects were transitioned to the same treatment regimen targeting BG<7.8 mmol/L before meals during the hospital stay and 90 days post discharge. The primary outcome was differences in a composite score of hospital complications including mortality, wound infection, pneumonia, bacteremia, respiratory failure, acute renal failure, and major cardiovascular events.

Results: A total of 302 patients were randomized to intensive (n=151) or conservative (n=151) glucose control following a computerized insulin infusion algorithm. The groups were well balanced. The mean ICU daily BG was 7.3±0.78 mmol/L (IQR 6.9–7.7) in the intensive group and 8.6±1.1 mmol/L (IQR 7.9–9.1) in the conservative group (p=0.99), and the hospital length of stay was 11.4±11 vs. 9.5±6 days (p=0.13), respectively. In the ICU, a BG <3.9 mmol/L occurred in 8% and 2% of the intensive and conservative groups (p=0.03), with no BG <2.2 mmol/L. After ICU care, there were no differences between intensive and conservative groups in mean daily BG (7.9±1.6 mmol/L vs. 7.8 ±1.6 mmol/L), patients with hypoglycaemia (<2.2 mmol/L: 1% vs. 3%, p=0.68), or hospital readmissions (18% vs. 20%, p=0.62).

Conclusion: In summary, intensive control targeting a BG of 5.6–7.8 mmol/L in the ICU did not reduce perioperative complications, mortality or hospital length of stay compared to a less strict glucose target of 7.8–10 mmol/L in hyperglycaemic patients undergoing CABG surgery.

Clinical Trial Registration Number: NCT 01792830

Supported by: American Diabetes Association, Sanofi and Glytec

OP 20 Novel compounds on the horizon

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Effect of omarigliptin, a novel once-weekly DPP-4 inhibitor, in Japanese patients with type 2 diabetes: a placebo- and sitagliptin-controlled trialI. Gantz¹, T. Okamoto², Y. Ito², K. Okuyama², S.S. Engel¹;¹Merck Sharp & Dohme Corp., Whitehouse Station, USA,²MSD K. K., Tokyo, Japan.

Background and aims: Omarigliptin (OMARI) is a potent, oral, once-weekly, dipeptidyl peptidase-4 (DPP-4) inhibitor in development for the treatment of type 2 diabetes mellitus. This is a randomized, double-blind, parallel-group, active and placebo-controlled study in monotherapy for Japanese patients with type 2 diabetes mellitus (T2DM) to assess the efficacy, safety, and tolerability of OMARI compared to placebo (PBO) and sitagliptin (SITA), a commonly used once-daily DPP-4 inhibitor.

Materials and methods: Patients either not on or on an oral antihyperglycemic agent (AHA) were eligible. After a diet/exercise run-in and, for patients on an AHA, a drug wash-off period, eligible patients (N = 414) were randomized (2:2:1) to OMARI 25 mg q.w., SITA 50 mg q.d. (recommended dose in Japan), or PBO for 24 weeks. The primary efficacy endpoint was the change in HbA1c from baseline at Week 24 with two co-primary hypotheses, superiority of OMARI to PBO and non-inferiority of OMARI to SITA based on the prespecified criterion of having an upper bound of the 95% confidence interval (CI) less than 0.3%.

Results: Treatment groups were well-balanced for baseline characteristics. At baseline, randomized patients had a mean HbA1c of 7.9, 8.0 and 8.1% in OMARI, SITA, and PBO group, respectively. Mean fasting plasma glucose (FPG) was also similar between treatment groups with 9.0, 8.8, and 9.0 mmol/L in OMARI, SITA and PBO group, respectively. At Week 24, OMARI significantly changed HbA1c by -0.80% from baseline relative to PBO (Table). The change (least squares mean, 95% CI) relative to SITA was -0.02% (-0.15, 0.12) and met the prespecified non-inferiority criterion. FPG and 2hr post meal glucose were significantly reduced from baseline with OMARI and SITA relative to PBO. OMARI was well tolerated without symptomatic hypoglycaemia and was not associated with weight gain.

Conclusion: In monotherapy, treatment with once-weekly OMARI provided comparable efficacy to once-daily SITA and was generally well tolerated over 24 weeks in patients with T2DM.

Glycemic Parameter	Omarigliptin q.w. (N=166)	Sitagliptin q.d. (N=164)	Placebo (N=82)
ΔHbA1c, %	-0.66 (-0.76, -0.57)†	-0.65 (-0.74, -0.55)†	0.13 (-0.00, 0.27)
ΔFPG, mmol/L	-1.03 (-1.21, -0.84)†	-1.15 (-1.34, -0.97)†	-0.35 (-0.60, -0.09)
Δ2hr postmeal glucose, mmol/L	-2.35 (-2.75, -1.96)†	-2.51 (-2.90, -2.12)†	-0.30 (-0.84, 0.23)

Data are expressed as least squares mean change from baseline (95% confidence interval).

†p<0.001 vs. placebo.

Based on a constrained Longitudinal Data Analysis (cLDA) model with terms for treatment, prior AHA therapy status (yes/no), time and the interaction of time by treatment, time by prior AHA therapy status, and time by treatment by prior AHA therapy status with the constraint that the mean baseline is the same for all treatment groups.

Clinical Trial Registration Number: NCT01703221

Supported by: MSD K. K.

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Postprandial effects of the phosphodiesterase-5 (PDE-5) inhibitor tadalafil in type 2 diabetes patients: a randomised controlled trial

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Background and aims: Type 2 diabetes (T2D) is characterised by postprandial hyperglycaemia and it is important to find better treatment for this metabolic aberration. One strategy may be to amplify NO signalling through PDE-5 inhibition which increases intracellular cyclic GMP levels. Previous data have shown that PDE-5 inhibition induced by tadalafil was able to im-

prove markers of insulin sensitivity in T2D patients. Here, we investigated acute metabolic and vascular effects of tadalafil in T2D patients after a mixed meal.

Materials and methods: We performed a randomised, double blind, placebo controlled trial, in parallel groups. Twenty-six T2D patients (Age: 40–70 (male) and 50–70 years (female); BMI: 27–40 kg/m²; HbA1c < 60 mmol/mol) were enrolled and they received either 20 mg tadalafil (n=14) or placebo (n=12) 30 min prior to a mixed meal. To assess forearm glucose uptake (FGU) and muscle capillary recruitment (PSglu), intramuscular microdialysis was combined with arterial and venous blood sampling, as well as plethysmography monitoring of forearm blood flow (FBF). Blood samples and tissue dialysates were repeatedly collected for 5 hours. We used an ANOVA and Mann-Whitney U-test for all statistical analyses. Furthermore, we performed an ad hoc analysis excluding patients on ACE-inhibitors because this family of drugs have known positive effects on glucose metabolism. Accordingly, 10 patients in the tadalafil group and 9 patients in the placebo group were evaluated in a subgroup analysis.

Results: The groups were comparable regarding gender, age, BMI and HbA1c. ITT analyses showed that T2D patients in the tadalafil group increased FBF ($p < 0.05$), whereas this was not observed in the placebo group. Moreover, incremental area under the curve (IAUC) for FGU and IAUC for PSglu during 5 hrs after the meal were similar in the two groups. However, the ad hoc analysis showed a significant increase ($p < 0.05$) for IAUC FGU as well as for IAUC PSglu ($p < 0.05$) in T2D patients treated with tadalafil (n=10) compared with placebo (n=9). Finally, postprandial glucose and insulin concentrations were similar in the two groups.

Conclusion: In conclusion, our data suggest that tadalafil may induce positive metabolic and vascular effects in the postprandial state in type 2 diabetes patients.

Clinical Trial Registration Number: NCT01238224

Supported by: Eli Lilly AB

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A selective GPR40 (FFAR1) agonist provides immediate and durable glucose control in rodent models of type 2 diabetes

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Background and aims: LY2881835 (LY) is a high affinity, potent, and efficacious GPR40 agonist when examined in GPR40 receptor binding and cell-based assays. In addition, a statistically significant (SS) increase in glucose-dependent insulin secretion was seen when LY was examined in primary mouse, rat and human islets. The following studies were performed to 1) explore the in vivo selectivity of LY using wild type (WT), GPR40 KO-, and GPR120-KO mice plus 2) the glucose lowering properties of LY in rodent models of type 2 diabetes.

Materials and methods: Islets isolated from the WT, GPR40 KO- and GPR120-KO mice were treated with LY2881835. Insulin concentrations were measured after drug incubation for 90 min. LY2881835 was administered orally to the WT, GPR40 KO- and GPR120-KO mice. An OGTT was performed 60 minutes following drug administration and immediately prior to administration of the oral glucose bolus. LY2881835 was administered orally once daily to DIO or Streptozotocin (STZ)-treated DIO mice for 14 days. OGTTs were performed on days 1, or 7 and 14 with the oral glucose bolus administered at 30 or 60 minutes following drug administration.

Results: A statistically significant (SS) increase in Insulin secretion was seen when LY was tested in primary islets from WT and GPR120 KO mice; although, no insulin secretion was detected in primary islets from GPR40 KO mice. OGTTs performed in WT, GPR40- and GPR120-KO mice following oral administration of LY at 30 mg/kg demonstrated SS reductions in glucose AUCs in WT and GPR120 KO mice but not in GPR40-KO mice when compared to controls. These findings demonstrate that LY induces specific GPR40-mediated anti-diabetic activity when examined ex-vivo and in-vivo. To explore the activity and the durability of LY in a model of insulin resistance, LY was administered orally, at 30 mg/kg to DIO mice (a model of Insulin resistance induced by a high fat/sucrose diet) for 14 days with OGTTs performed on days 1 and 14. SS reductions in glucose AUCs were seen on days 1 and 14. A similar study was performed in STZ-treated DIO mice to explore glucose control in a model of type 2 diabetes. In this model, pancreatic insulin content was reduced ~80% due to STZ-treatment. Glucose AUCs were SS reduced during OGTTs performed on days 1, 7 and 14 compared to control mice.

Conclusion: These results demonstrate that LY functions as a GPR40-specific agonist mediating immediate and durable glucose control in rodent models of type 2 diabetes. The findings suggest that GPR40 agonism could benefit glucose control across the spectrum of type 2 diabetes phenotypes including those patients with insulin resistance and substantially reduced beta-cell function.

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Pramlintide-insulin fixed-dose combination: a phase 1 dose ratio-finding study in patients with type 1 diabetes mellitus

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Background and aims: Patients with type 1 diabetes mellitus (T1DM) lack secretion of both insulin and amylin, which are normally co-secreted by β -cells; thus, replacing both hormones may provide therapeutic advantages in optimizing glycaemic control. In treating T1DM, fixed doses (e.g. 60 μ g) of pramlintide (an analog of human amylin) are administered as adjunct to prandial insulin, irrespective of insulin dose. This phase 1 study adjusted the pramlintide dose relative to insulin and examined the effects of 3 dose ratios on postprandial glucose and glucagon for 3 hours after a standard breakfast (600 kcal, 55% carbohydrate, 15% protein, 30% fat) meal.

Materials and methods: Subjects in this single-blinded, 4-way, crossover study received regular insulin + placebo, pramlintide 6 μ g/U + regular insulin, 9 μ g/U + regular insulin, and 12 μ g/U + regular insulin in random order, using a 30% reduction in usual preprandial insulin dose with an allowed maximum of 10U in all treatment groups before the test meal. Adverse events were identified on non-directed questioning, laboratory values, vital signs, and physical exam.

Results: Of 19 subjects randomized (mean [SD] age 46.2 [15.6] years, HbA1c 7.75 [0.58] %, weight 81.5 [11.1] kg, BMI 26.4 [2.6] kg/m²), 17 completed all four treatments. Premeal fasting plasma glucose levels ranged from 7.7 (2.0) to 8.7 (2.0) mmol/L, and insulin doses from 5.1 (1.54) to 5.4 (1.28) U across all groups. Mean (SEM) postprandial incremental glucose AUC_{0-3h} (mmol/L·h) for the pramlintide 6, 9, and 12 μ g/U + regular insulin groups were 456.6 (102.7), 476.7 (103.5), and 321.3 (99.8), respectively, versus 1142.5 (99.7) for placebo. Postprandial incremental glucagon AUC_{0-3h} (ng/L·h) for the pramlintide 6, 9, and 12 μ g/U + regular insulin groups were 649.4 (342.5), 614.9 (345.0), and 677.7 (333.8), respectively, versus 1503.0 (334.2) for placebo. All three pramlintide dose ratios lowered incremental glucose and glucagon AUC 58–72% and 55–59%, respectively, versus placebo. No treatment-related hypoglycaemia was reported. One subject reported nausea in all 3 pramlintide dose ratios, and 1 reported abdominal pain and diarrhoea.

Conclusion: All three pramlintide dose ratios showed efficacy in lowering postprandial glucose and glucagon levels, and were generally well-tolerated.

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A novel chemically modified analogue of xenin-25 exhibits improved glucose-lowering and insulin-releasing actions

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Background and aims: Xenin-25 is a K-cell derived gut peptide that is co-secreted with glucose-dependent insulinotropic polypeptide (GIP) following a meal. Recent studies have shown that xenin-25 improves glucose homeostasis and possibly potentiates the biological action of GIP. However, like many regulatory gut peptides, xenin-25 is rapidly degraded by plasma enzymes following release into the circulation. The aim of the present study was to examine the biological actions of a novel analogue of xenin-25, chemically modified by substitution of naturally occurring Lys and Arg residues with Gln, namely xenin-25(Gln).

Materials and methods: Xenin-25 and xenin-25(Gln) were synthesised by Fmoc peptide chemistry, purified by rp-HPLC and characterised by MALDI-ToF MS. Xenin-25 and xenin-25(Gln) were incubated with murine plasma in 50 mmol/l TEA/HCl buffer (pH 7.8) at 37°C for 0, 2, 8 and 24 hours and degradation profiles analysed by rp-HPLC and MALDI-ToF MS. *In vitro* insulin-

releasing properties of xenin-25(Gln) (10^{-12} to 10^{-6} mol/l) were determined in BRIN-BD11 cells ($n=8$; 20 min incubation) at 5.6 and 16.7 mmol/l glucose concentrations in the absence and presence of (d-Ala²)GIP. Acute effects of xenin-25(Gln) (25 nmol/kg bw; *ip*) on plasma glucose and Insulin concentrations were examined in NIH Swiss mice fed a high fat diet (45% fat, 35% carbohydrate and 20% protein) for 140 days prior to experiment. Persistent effects of xenin-25(Gln) on blood glucose concentrations were examined in lean C57Bl/6J mice.

Results: Xenin-25(Gln) was resistant to plasma-mediated degradation for up to 24 hours whereas native xenin-25 was sequentially degraded by murine plasma. In BRIN-BD11 cells, xenin-25(Gln) significantly stimulated (1.5 to 2.9-fold; $P < 0.05$ to $P < 0.001$) Insulin secretion in a concentration-dependent manner at both 5.6 and 16.7 mmol/l glucose. Moreover, xenin-25(Gln) significantly augmented (1.4 to 1.7-fold; $P < 0.05$ to $P < 0.01$) the insulinotropic response of (d-Ala²)GIP *in vitro*. Acute administration of xenin-25(Gln) to high fat fed mice significantly reduced plasma glucose concentrations compared to glucose alone (56% reduction; $P < 0.001$) and xenin-25 (38% reduction; $P < 0.05$) treated mice. Correspondingly, the overall Insulin secretory response was significantly enhanced (3.4-fold increase; $P < 0.05$) in xenin-25(Gln) treated mice compared to high fat fed mice administered glucose alone. Furthermore, xenin-25(Gln) elicited a protracted glucose-lowering effect (40% reduction; $P < 0.001$) when administered 8 hours prior to a glucose load in C57Bl/6J mice.

Conclusion: In summary, xenin-25(Gln) represents a novel enzymatically stable analogue of xenin-25 with improved glucose-lowering and insulinotropic actions over the native peptide. As a result, xenin-25(Gln) can be positioned as a novel treatment of type 2 diabetes.

Supported by ERDF/INI

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Faster-acting insulin aspart improves postprandial glycaemia versus insulin aspart in patients with type 1 diabetes mellitus

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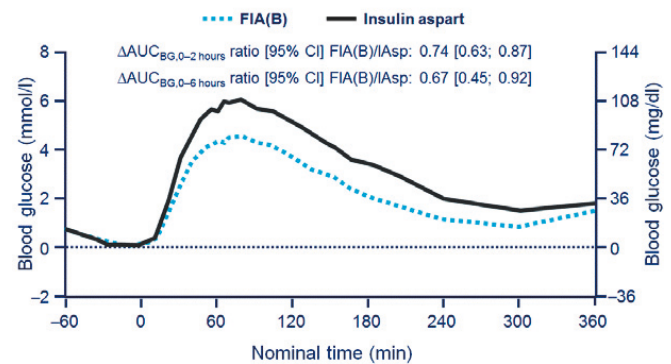
Background and aims: Faster-acting insulin aspart is insulin aspart (IAsp) in a new formulation containing two new excipients (nicotinamide and arginine), which result in a faster initial absorption after s. c. injection.

Materials and methods: In total, 36 patients with type 1 diabetes mellitus (T1DM; mean \pm standard deviation age: 38.6 ± 12.5 years; haemoglobin A_{1c}: $7.8 \pm 0.81\%$) received a single dose (0.2 U/kg s. c.) of faster-acting insulin aspart formulation 'B' (FIA(B)) or IAsp immediately (≤ 2 minutes) before a standardised liquid meal test (600 kcal; 67% carbohydrates, 17% protein, 16% fat; to be consumed within 8 minutes) in a randomised, crossover design.

Results: Compared with IAsp, FIA(B) had a faster onset of appearance, i.e., time from trial drug administration until the first time serum insulin aspart concentration was >30 pmol/l (median difference [95% confidence interval (CI)]: -6.6 minutes [-8.0; -5.0]) and greater exposure during the first 2 hours with the largest difference in the first 15 minutes, whereas the overall pharmacokinetic exposure was similar between the two insulins (mean ratio [95% CI] AUC_{0-15 minutes}: 3.14 [2.59; 3.80]; AUC_{0-30 minutes}: 1.93 [1.64; 2.26]); AUC_{0-1 hour}: 1.30 [1.15; 1.46]; AUC_{0-2 hours}: 1.13 [1.03; 1.24]; AUC_{0-10 hours}: 0.99 [0.93; 1.06]). The faster absorption led to a greater reduction in postprandial blood glucose (BG) with FIA(B) versus IAsp, indicated by lower post-meal AUC_{BG} over 2 and 6 hours (reduction by 26% and 33%, respectively; Figure), and by lower BG values 1 and 2 hours postprandially (mean difference [95% CI] BG_{1 hour}: -1.24 mmol/l [-2.01; -0.46]; BG_{2 hours}: -1.45 mmol/l [-2.49; -0.42]). No safety or tolerability issues were identified; in particular, no injection site reactions were observed.

Conclusion: FIA(B) was absorbed faster and had an increased early exposure than insulin aspart, leading to improved postprandial glycaemic control versus insulin aspart.

Figure: Meal test blood glucose profile.



Clinical Trial Registration Number: NCT01121276

Supported by: Novo Nordisk

OP 21 Physiological adaptation to bariatric surgery

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Bariatric surgery improves whole body and femoral subcutaneous adipose tissue insulin sensitivity independently of whole body weight loss

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Background and aims: The effect of weight reduction by dietary treatment does not reverse adipose tissue insulin resistance, as opposed to skeletal muscle in which it makes a big improvement. To the best of our knowledge, there is no evidence of how bariatric surgery modulates femoral adipose tissue glucose uptake. We examine how bariatric surgery modifies femoral adipose tissue glucose uptake both in fasting condition and during euglycemic hyperinsulinemic clamp in severely obese patients and healthy lean subjects and we study if the changes in adipose tissue glucose uptake were related with the remission of diabetes.

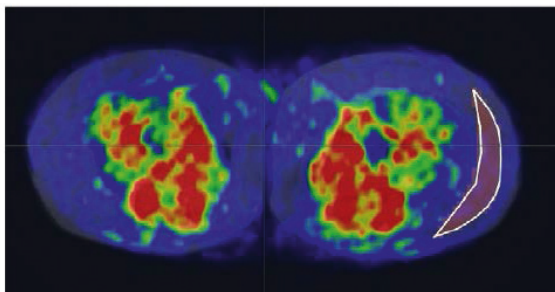
Materials and methods: Femoral subcutaneous adipose tissue and muscle glucose uptake were studied with positron emission tomography using [¹⁸F] fluorodeoxyglucose (Figure 1) during fasting and euglycemic hyperinsulinemic clamp in 25 morbidly obese patients (BMI of 43.2±3.6 kg/m²) before and six months after the bariatric surgery. Ten age-matched lean subjects (BMI: 23.7±1.8 kg/m²) served as controls. All data are presented as mean ± SE.

Results: At baseline, nine patients had type 2 diabetes mellitus (T2DM) and seven had impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). After surgery, remission of T2DM or IFG/IGT was observed in ten patients. Two patients dropped out from the study. At baseline, obese patients had impaired whole body (M value: 12.2±1.14 vs 40.3±3.00 μmol/(min*kg), p<0.001) and tissue specific (muscle 20.0±3.24 vs 68.4±8.24 μmol/(min*kg), p<0.001 and adipose tissue 5.8±0.54 vs 9.3±1.40, p<0.01) insulin sensitivity compared to controls. After the bariatric procedure, obese patients lost 23±6% of body weight. M value and muscle and adipose tissue glucose uptake were increased by 106±80%, p<0.001; 166±173%, p<0.001; 77±129%, p=0.03, respectively. Furthermore, adipose tissue glucose uptake normalised after surgery. There were no differences in fasting adipose tissue glucose uptake before or after surgery in obese as compared to controls. Neither the changes in whole body nor the changes in tissue specific insulin sensitivity correlated with the change in weight loss. The remission of diabetes was positively correlated with the change produced by bariatric surgery on the effect of insulin on adipose tissue glucose uptake (Spearman's correlation: R=0.54; p<0.05).

Conclusion: Bariatric surgery promotes the remission of T2DM and insulin resistance and this is connected with the improvement of insulin action on adipose tissue glucose uptake. Improved whole body and femoral subcutaneous adipose tissue insulin sensitivity do not relate with the whole body weight loss. Hence, insulin sensitivity may depend on the intrinsic action of the bariatric surgery.

Figure 1. Positron emission tomography image overlaid to a magnetic resonance image.

A region of interest has been drawn on the femoral subcutaneous adipose tissue.



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Supported by: Academy of Finland, Sigrid-Juselius Foundation and HEPADIP EU FP6

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Meal protein uptake and systemic leucine kinetics after Roux-en-Y gastric bypass

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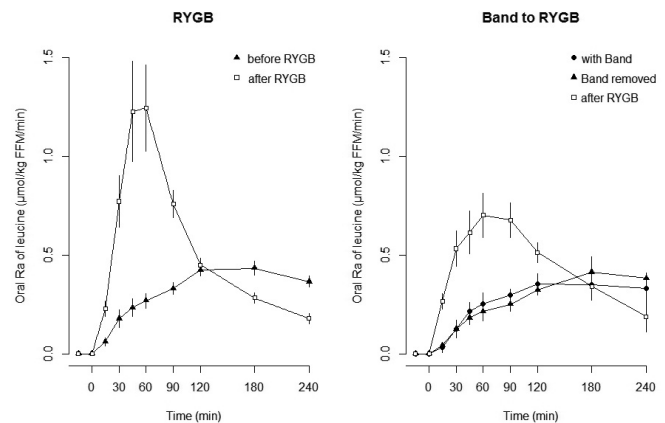
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Background and aims: Gastric emptying and glucose absorption is accelerated after Roux-en-Y gastric bypass (RYGB), but absorption kinetics of other nutrients are unknown. Exclusion of major parts of the stomach and delayed admixing of nutrients and pancreatic proteases could potentially impair protein digestion and absorption after surgery.

Materials and methods: Leucine kinetics was investigated after meal ingestion in 9 obese glucose tolerant subjects (BMI 39.2±1.8 kg/m² (mean±sem), HbA1c 5.3±0.1%) before and 3 months after primary RYGB. Additionally, 5 obese subjects (BMI 42.1±1.7 kg/m², HbA1c 5.4±0.1%) who underwent conversion of gastric band to RYGB were studied with the band, after band removal and 3 months after conversion to RYGB. All received a 6 h primed-continuous infusion of [3,3,3-²H₃]-leucine combined with a 4 h meal test (200 mL, semi-liquid, 394 kcal, carbohydrate 50%, fat 35% and protein 15%) containing 15.2 g of casein intrinsically labeled with [1-¹³C]-leucine. Fat free mass (ffm) was assessed by whole body DEXA scan.

Results: Plasma leucine concentration was unchanged at fasting while the postprandial leucinemia increased (iAUC: pre 4.1±0.6, 3 mo 9.5±2.0 mmol/L×min, p=0.03) but was shorter lasting after RYGB. Peak rate of appearance (Ra) of oral leucine increased (0.45 ± 0.04 vs 1.35 ± 0.22 μmol/kg ffm/min, p<0.01) and occurred earlier (173 ± 16 vs 65 ± 8 min, p<0.01) and oral recovery increased over the 4 h measured (49±4% vs 70±4%, p<0.01). Peak rate of disappearance (Rd) of leucine increased after RYGB (1.9 ± 0.07 vs 2.9 ± 0.26 μmol/kg ffm/min, p<0.01). Band removal did not change oral Ra (peak: 0.44 ± 0.06 vs 0.44 ± 0.07 μmol/kg ffm/min), oral recovery (38±4% vs 40±4%) or Rd of leucine (peak: 1.9 ± 0.2 vs 1.8 ± 0.1 μmol/kg ffm/min), but similar increases were seen after conversion to RYGB as after primary RYGB.

Conclusion: In conclusion, digestion and absorption of casein are dramatically changed after RYGB from slow to fast, while protein kinetics is not altered by the gastric band. Protein digestion and absorption thus seem to be accelerated rather than impaired after RYGB. Although the nutritional consequences are unknown, fast absorption could change the nutritional anabolic effect of protein potentially favoring oxidation rather than protein synthesis. These findings may have implications for our recommendation of source, quantity and frequency of protein intake after RYGB.



Clinical Trial Registration Number: NCT01559792

Supported by: UNIK

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Beta cell function improvements in subjects with type 2 diabetes 1 year after biliopancreatic diversionA.C.J. Vasques¹, J.C. Pareja², M.S. Oliveira², E.S. Novaes², M.M.O. Lima², É.A. Chaim³, F. Piccinini⁴, C. Dalla Man⁴, C. Cobelli⁴, B. Geloneze²;¹Laboratory of Investigation on Metabolism and Diabetes (LIMED), School of Applied Sciences, Limeira, ²Laboratory of Investigation on Metabolism and Diabetes (LIMED), Faculty of Internal Medicine, Campinas,³Department of Surgery, Faculty of Internal Medicine, Campinas, Brazil,⁴Department of Information Engineering, University of Padua, Italy.

Background and aims: Bariatric surgery is an alternative therapeutic approach for obese patients with poorly controlled type 2 diabetes (T2DM). To provide evidence for the underlying mechanisms associated with T2DM remission after biliopancreatic diversion (BPD), this study aimed to evaluate the long term effect of BPD on β -cell function parameters and insulin sensitivity in grade I and II obese patients with T2DM.

Materials and methods: Sixty-eight women were divided into three groups: 19 LeanNGT (BMI: 23 ± 2 kg/m²), 18 ObeseNGT (BMI: 35 ± 5 kg/m²) both with normal glucose tolerance (NGT), and 31 ObeseT2DM (BMI: 36 ± 4 kg/m²). Twenty ObeseT2DM women underwent BPD and were reassessed 1 year after surgery. OGTTs and hyperglycaemic clamps were performed. Mathematical modeling, based on insulin and C-peptide serum levels, was used to analyze β -cell function, insulin sensitivity (IS_{oral} and IS_{clamp}) and delay time of β -cell response to a specific plasma glucose concentration. The basal (DI_b), dynamic (DI_d), static (DI_s) and total disposition indexes (DI_{oral} and DI_{clamp}) represents β -cell function adjusted to IS and were calculated by multiplying β -cell responsivity indices by IS.

Results: After BPD, BMI (pre: 36 ± 4 vs post: 28 ± 3 kg/m²), fasting glycaemia (pre: 133 ± 38 vs post: 88 ± 13 mg/dl) and HbA1c levels (pre: 7.2 ± 1.3 vs post: $5.1 \pm 0.9\%$ were reduced ($p < 0.001$). At baseline, IS_{oral} and IS_{clamp} were reduced in the obese groups compared with the LeanNGT group ($p < 0.001$). After BPD, IS indexes increased approximately 6-fold. IS_{oral} became comparable to LeanNGT, and IS_{clamp} became increased in comparison with the LeanNGT [29 (9–89) vs 15 (8–24) dl/kg/min per μ U/ml, $p < 0.01$], respectively. At baseline, the basal disposition index (DI_{basal}) was reduced in the ObeseT2DM [67 (44–145) dl/kg/min² per pmol/l] compared with the NGT groups [Lean: 258 (170–455) and Obese: 169 (119–326) dl/kg/min² per pmol/l; $p < 0.001$]. After surgery, the DI_{basal} increased 3-fold reaching similar levels to that of both NGT groups ($p = 0.167$). At baseline, the DI_d, DI_s and DI_{oral} derived from the OGTT were reduced in the ObeseT2DM compared with both NGT groups ($p < 0.001$). After surgery, DI_d and DI_{oral} were similar to ObeseNGT and remained reduced compared with the LeanNGT, while the DI_s was restored to the levels of both NGT groups. In the clamp test, at baseline, DI_d, DI_s and DI_{clamp} of the ObeseT2DM were similar to ObeseNGT and reduced compared with the LeanNGT ($p < 0.05$). After surgery, all of the DI were similar to LeanNGT levels and were increased compared with the ObeseNGT. The delay time, at baseline, was markedly increased in the ObeseT2DM compared with the NGT subjects in both dynamic tests ($p < 0.001$). After BPD, the delay time was decreased (Clamp: 100 (80–108) vs 29 (8–100) min.; OGTT: 15 (9–74) vs 9 (7–10) min.; $p < 0.05$) and reached similar levels to both NGT groups ($p = 0.8$).

Conclusion: In long term, the BPD resulted in positive physiological adaptations in patients with grade I and II obesity and T2DM, with improvements in a wide range aspects of β -Cell function and IS which probably contributed to the improved glycaemic control.

Clinical Trial Registration Number: U1111-1137-0489

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Restoration of beta cell function in severely obese type 2 diabetic patients after gastric bypass surgery is accompanied by improved insulin processingE. Svehlikova¹, A. Tuca¹, V. Höller¹, B. Obermayer-Pietsch¹, O. Freisinger², F. Tadler³, B. Ernst⁴, B. Wilms⁵, M. Thurnheer⁴, B. Schultes⁴, T.R. Pieber¹;¹Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, ²Department of Surgery, Medical University of Graz, ³Department of Surgery, Krankenhaus der Elisabethinen, Graz, Austria, ⁴Interdisciplinary Obesity Center, eSwiss Medical & Surgical Center, St. Gallen, ⁵Exercise Physiology Lab, Institute of Human Movement Sciences, ETH Zurich, Switzerland.

Background and aims: Gastric bypass surgery improves glycaemic control, but the underlying mechanisms are incompletely understood. The aim of the study was to quantify changes in beta cell function and insulin sensitivity before, early and 1 year after gastric bypass in type 2 diabetic (DM) and non-diabetic (ND) morbidly obese patients.

Materials and methods: Before, 8 to 21 days and 1 year after the surgery, 34 T2DM (17 with diabetes duration ≥ 8 years) and 21 ND patients underwent an oral glucose tolerance test (OGTT) and a botnia clamp combining an intravenous glucose tolerance test (IVGTT) with a subsequent hyperinsulinaemic-euglycaemic clamp.

Results: In DM patients, glucose tolerance gradually improved after the surgery, fasting insulin was decreased at 1 year ($p < 0.001$). In the OGTT total insulin secretion (AUC_{CP}/AUC_{GLU} : time $p < 0.001$; group $p < 0.001$) as well as the early insulin response (time $p < 0.001$; group $p < 0.001$) displayed an early improvement in both groups followed by decline or constant values respectively. DM patients did not reach non-diabetic values, but they showed an improved C-peptide secretion pattern with a shorter time to peak C-peptide ($p < 0.01$) and reduced late hyperinsulinaemia in OGTT. Insulin response to the IVGTT (AIR) did not change early after the surgery, but declined at 1 yr (time $p < 0.001$; group $p < 0.001$). On the contrary, proinsulin levels during OGTT successively declined after the surgery in both groups (peak proinsulin: time $p < 0.01$). The proinsulin/C-peptide ratio decreased early postoperatively and remained comparable at 1 yr in both groups (time $p < 0.001$). Having higher baseline values, DM group showed a greater reduction in proinsulin/C-peptide ratio compared to ND (time \times group $p < 0.01$). Insulin sensitivity displayed an early increase in DM patients reaching non-diabetic values at 1 yr and a later improvement in the ND group (Glucose disposal: time $p < 0.001$; group $p < 0.001$). The disposition index increased in DM patients but declined in ND group (time \times group $p < 0.01$; group $p < 0.001$).

Conclusion: The progressive improvement of glucose metabolism in DM patients after gastric bypass surgery relies on both improved insulin sensitivity and beta-cell function that however does not normalize entirely. Importantly, our results on proinsulin levels provide the first and strong evidence of improved insulin processing after gastric bypass, which may represent an important mechanism of the improvement of beta cell function in DM patients after the surgery.

	DM			ND		
	preop.	early	1 yr	preop.	early	1 yr
AUC_{CP}/AUC_{GLU}	0.4 ± 0.06	0.7 ± 0.08	0.5 ± 0.04	1.0 ± 0.06	1.2 ± 0.07	1.0 ± 0.07
Early insulin response	0.1 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.5 ± 0.06	0.64 ± 0.07	0.64 ± 0.07
Proinsulin (peak) (μ U/ml)	88.6 ± 13.7	63.8 ± 7.3	36.8 ± 4.2	87.3 ± 14.7	83.2 ± 12.4	58.5 ± 10.0
Proinsulin / C-peptide (peak)	16.3 ± 1.4	8.5 ± 0.9	6.6 ± 0.9	11.1 ± 1.0	7.1 ± 0.6	7.9 ± 1.6
Glucose disposal ($mg \cdot kg^{-1} \cdot min^{-1}$)	2.1 ± 0.3	5.5 ± 1.2	8.6 ± 0.6	7.9 ± 0.7	7.2 ± 0.5	11 ± 0.6
Disposition index	254 ± 69	672 ± 237	657 ± 127	2094 ± 323	1764 ± 296	1533 ± 194

Tab.: Selected indices of beta cell function, insulin sensitivity and serum proinsulin concentrations. Data shown as mean \pm SEM. Statistical significance in text.

Clinical Trial Registration Number: NCT 01271062

Supported by: EFSD/MSD

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Mechanisms of post-prandial hypoglycaemia after Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (LSG)

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Background and aims: Symptomatic hypoglycaemia (Hypo) is a well recognised complication of RYGB surgery. Data on the development of postprandial Hypo after LSG are scanty. We investigated the mechanisms of postprandial Hypo in patients undergoing RYGB or LSG.

Materials and methods: 32 obese non-diabetic subjects treated with RYGB and 39 with LSG received a 3-h OGTT before and 12–18 months after surgery. Hypo was defined as plasma glucose ≤ 2.7 mmol/L. Insulin sensitivity was assessed by OGIS index and β -cell function by modelling analysis of the C-peptide response to glucose load.

Results: Postprandial Hypo occurred in 20 of 32 RYGB patients and in 13 of 39 LSG patients. Age did not discriminate Hypo from non-Hypo (NH) subjects. Presurgery BMI was lower in RYGB-Hypo than RYGB-NH (43.8 ± 5.2 vs 49.7 ± 6.1 kg/m², $p=0.004$), but not in LSG-Hypo vs LSG-NH patients. Similarly, baseline insulin sensitivity was higher in RYGB-Hypo than RYGB-NH (386 ± 53 vs 325 ± 44 ml.min⁻¹.m⁻², $p=0.004$), but was similar in LSG-Hypo and LSG-NH subjects. After either operation, insulin sensitivity improved ($p<0.0001$) to the same extent in Hypo and NH subjects. Pre-surgery fasting glycaemia was lower in both RYGB-Hypo and LSG-Hypo compared to the respective NH group (5.0 ± 0.4 vs 5.7 ± 0.9 mM, $p<0.001$, and 5 ± 0.3 vs 5.6 ± 0.7 mM, $p<0.02$ in LSG). Similarly, before surgery mean OGTT glycaemia was lower in Hypo than NH subjects (6.9 ± 0.9 vs 8.4 ± 1.3 mM, $p=0.001$), as was the plasma glucose nadir (4.7 ± 1.3 vs 6.4 ± 1.6 mM, $p=0.005$) similarly in the two groups. Pre-surgery fasting plasma insulin and insulin secretion rate were similar at baseline in all four groups, and were similarly reduced after surgery ($p<0.0001$). In contrast, total insulin secretion was reduced in NH, but not in Hypo after either intervention (79 ± 23 vs 59 ± 27 and 71 ± 28 vs 72 ± 30 nmol.m⁻², $p<0.01$ for the timexgroup interaction). β -cell glucose sensitivity was negatively correlated with glucose nadir values in both surgeries ($p=0.02$ in RYGB and $p=0.0009$ in LSG). Likewise, insulin sensitivity was inversely correlated with the glucose nadir ($p<0.0001$ in RYGB and $p=0.009$ in LSG).

Conclusion: Baseline lower plasma glucose concentrations during an OGTT are associated with a higher risk of post-surgery reactive hypoglycaemia after both RYGB and LSG. Mechanistically, inherently higher insulin output and better β -cell glucose sensitivity and peripheral insulin sensitivity are responsible for postprandial Hypo in surgically weight-reduced patients.

36.7 ± 2.7 kg/m²). It was our aim to modify eating behavior and dumping-like postprandial glucose excursions. During one week of sensor-augmented nutrition counseling, participants were asked to comply with defined nutrition targets ($<30\%$ fat/ $>40\%$ carbohydrates/ <20 g sucrose/ >25 g fiber) and record nutrition and physical activity in detailed diaries.

Results: Daily energy intake (1409 ± 135 kcal; mean \pm SEM) was hypocaloric, whereas fat consumption exceeded general recommendations both in absolute (64 ± 7 g) and relative terms (42% of daily energy intake). Carbohydrate ingestion was slightly too low both in absolute (131 ± 15 g) and relative terms (38% of daily energy intake), however 21% of the carbohydrates were consumed as sucrose (28g). Protein intake (69 ± 1 g / 20% of daily energy intake) was rather low and fiber consumption (13 ± 2 g) definitely too low. In two female subjects, one with (DM+) and one without preoperative diabetes (DM-), complete CGM-datasets before and after nutrition training have yet been compiled: Target adherence reduced glucose variability (STD) from 45 to 31 (DM-) and from 24 to 16 (DM+), respectively; time spent in hypoglycaemia (IG <70 mg/dl) decreased from 11 to 8.4% (DM-) and from 29 to 2.6% (DM+) of the day, respectively; time spent in hyperglycaemia (IG >180 mg/dl) decreased from 8.9 to 3.2% (DM-) and from 0.9 to 0% (DM+) of the day.

Conclusion: Inadequate postbariatric eating behavior was prevalent in symptomatic NIPHS subjects. In contrast to bariatric literature, sucrose and fat consumption were exceeding and fiber and complex carbohydrates falling short of prescribed and desirable amounts. Sensor augmented nutrition training focusing on strict adherence to adequate nutrition targets markedly reduced prandial glucose peaks, overall glucose variability and time spent <70 mg/dl in two preliminary cases. We challenge the idea of RYGB-induced prudent food preference. Sensor augmented nutrition training may help to avoid sweetened food as far as possible in order to balance accelerated glucose appearance after RYGB.

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Inadequate eating behaviour and accelerated glucose appearance can be resolved by sensor-augmented nutrition training in patients with hypoglycaemias (NIPHS) after RYGB

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Background and aims: Gastric bypass (RYGB) has been said to promote prudent eating by altering food preference and reducing intake of fat and sweet foods thereby contributing to the favourable effects on metabolic control and body weight. On the other hand, recent clinical and experimental data suggest accelerated appearance of oral glucose due to the anatomic alterations and metabolic reprogramming of the Roux limb. We therefore assessed both eating behavior and glucose excursions in RYGB subjects with noninsulinoma pancreatogenous hypoglycaemia syndrome (NIPHS) - a condition with high glucose variability - under real life conditions.

Materials and methods: In 20 patients (18 f, 2 m) who had undergone RYGB for obesity (BMI 45.5 ± 2.2 kg/m²; 80% type 2 diabetes) and in whom both NIPHS and inadequate postbariatric eating behavior had been diagnosed during a week of continuous glucose monitoring (CGM; Dexcom G4, lower alarm limit: 70 mg/dl) 6–18 months after the bariatric procedure (BMI

OP 22 Neuropathy: mechanisms and outcomes

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Diabetic neuropathy and urologic complications in men with type 1 diabetes in the DCCT/EDIC study

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Background and aims: Impaired genital sensory or motor function may promote sexual or urinary dysfunction, but data in persons with type 1 diabetes (T1D) are limited. We evaluated associations between diabetic peripheral neuropathy (DPN), erectile dysfunction (ED) and lower urinary tract symptoms (LUTS) in men with T1D participating in Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications (DCCT/EDIC).

Materials and methods: DPN was evaluated with a composite of symptoms, neurological examination and nerve conduction studies (NCS) performed at baseline and biennially during DCCT, and at years 13/14 during EDIC. Confirmed DPN was defined as NCS abnormalities in ≥ 2 nerves and ≥ 2 positive responses among symptoms, sensory signs, or reflex changes. ED was assessed using the question from the International Index of Erectile Function “Over the past 4 weeks, how would you rate your confidence that you can get and keep erection?” at EDIC year 17. Answers “very low-low” were classified as ED. LUTS was assessed by the American Urological Association Symptom Index (AUASI). AUASI scores 0–7 were defined as no LUTS, and AUASI of 8–35 as LUTS.

Results: ED and LUTS data were available from 635 men (mean age 52 years, mean duration 30 years, mean DCCT/EDIC A1c 7.9%). ED only was reported by 193 (30%), LUTS only by 61 (10%) and both ED and LUTS by 97 (15%) men. Men with confirmed DPN at year 13/14 were more likely to report ED (41%), LUTS (31%) or both ED and LUTS (62%) at EDIC year 17 compared to those without ED or LUTS (22%) ($p < 0.0001$ for all). In multivariable logistic regression analysis adjusting for DCCT cohort assignment (Intensive vs. Conventional), DCCT/EDIC HbA1c, DCCT/EDIC systolic blood pressure, age, smoking and drinking status, men with confirmed DPN had 3.82 (95% CI 2.0–7.3) greater odds of having both ED and LUTS compared to those without DPN.

Conclusion: Men with T1D and DPN are more likely to report urologic complications than men with no DPN. The temporal relationship between DPN and ED/LUTS in this cohort is under analysis.

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Erectile dysfunction and health-related quality of life in type 1 diabetes: longitudinal follow-up of the DCCT/EDIC cohort

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Background and aims: No longitudinal studies have examined the effects of erectile dysfunction (ED) on health-related quality of life (HRQOL) among men with type 1 diabetes (T1DM). We used biomedical information gathered as part of the Diabetes Control and Complications Trial and its follow-up the Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study to examine the relationship.

Materials and methods: Assessment of ED was made annually in EDIC through a question asking “Has the patient experienced impotence since the last completed annual visit?” The Diabetes Quality of Life Scale (DQOL) (higher score, better QOL; scale 0–100), was given biannually throughout

EDIC follow-up. The cumulative effects of ED over the 17-year time-frame were examined by calculating the number of consecutive years of ED as of year 17. A multivariable regression model was built to estimate the association between ED experience and having a low DQOL score (≤ 25 th percentile; DQOL = 70). Analyses were adjusted for time-weighted DCCT/EDIC HbA1c levels.

Results: At EDIC baseline, the 693 male subjects with ED and QOL data were on average 35.6 ± 6.7 years old with a mean BMI of 26.3 ± 3.6 kg/m², diabetes duration 13.3 ± 4.8 years, and DCCT/EDIC time-weighted HbA1c of $8.1 \pm 1.3\%$. Forty-one (6%) subjects reported ED at baseline (ED mean DQOL 69.6 ± 10.3 vs. No ED 76.9 ± 9.2 ; $p < 0.0001$) and 232 (37%) reported ED at EDIC year 17 (ED mean DQOL 72.6 ± 11.2 vs. No ED 77.7 ± 10.5 ; $p < 0.0001$). Among the 652 men who reported no ED at baseline, 332 (51%) went on to develop ED; 262 (79%) of the incident cases were reported at 2 or more follow-up visits. The mean DQOL at year 17 decreased with the number of consecutive years of ED (mean DQOL score for no ED 77.7 ± 10.5 , 1 year of ED 75.3 ± 13.4 , 2 years of ED 71.9 ± 9.5 , 3 years of ED 73.1 ± 8.6 , and 4 or more years of ED 71.8 ± 11.1 ; $p < 0.0001$). The table shows the effect of consecutive years of ED as of year 17 on the odds of a low total DQOL score at year 17 after adjusting for HbA1c. While HRQOL decreases initially with increased duration of ED, these effects start to diminish as the number of years with ED increases, suggesting some adaptation to the change in health state.

Conclusion: Development of ED is common in T1DM patients and is associated with decrease in health-related quality of life. The effects however are not cumulative as men may begin to cope with the change in health state.

Adjusted Odds Ratios (95% CI) of a Low DQOL Score (≤ 25 th percentile) by ED Experience

	OR (95% CI)
Consecutive years of ED as of year 17	
1	2.29 (1.14, 4.60)
2	3.66 (1.63, 8.20)
3	2.59 (0.92, 7.27)
4+	2.64 (1.70, 4.11)
Time-weighted DCCT/EDIC HbA1c (%)	1.24 (1.02, 1.51)

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Diabetic peripheral neuropathy adversely affects balance during stair ascent and descent

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Background and aims: Patients with diabetic peripheral neuropathy (DPN) are known to display unsteadiness during walking and as a result be at increased risk for falling. Whilst some studies have found increased postural sway during quiet standing and walking on level ground in patients with DPN, no data exist on objective measures of balance during stair walking. Walking on stairs is one of the most dangerous daily activities in terms of fall risk and this study investigated the underlying mechanisms of unsteadiness in patients with DPN during stair ascent and descent.

Materials and methods: Motion and force data were collected for 22 diabetes patients with DPN (PN; age: 57 ± 9.3 years), 40 diabetes patients with no DPN (DM; 57 ± 12.8 years), and 32 healthy controls (Ctrl; 50 ± 19 years). Movement data was collected using a 10-camera 3D motion analysis system from reflective markers placed at anatomical locations on the body to calculate whole-body centre-of-mass (CoM). The centre-of-pressure (CoP) under the feet was measured using 4 force platforms mounted into the middle of a 7-step staircase, which participants ascended and descended at least 3 times. Balance was quantified by assessing the separation between the centre-of-mass and centre-of-pressure (CoM-CoP separation) in the medial-lateral plane. This parameter was expressed in terms of maximum separation and the variation in separation (standard deviation within 3 trials). Results were analysed using analysis of variation with Tukey post-hoc tests.

Results: During stair ascent the PN group showed significantly higher maximum CoM-CoP separation (mean [SD] PN: 13 [2], DM: 10 [3], Ctrl: 10 [3] cm; $p < 0.01$) and significantly increased variation in CoM-CoP separation (mean [SD] PN: 7 [1], DM: 5 [1], Ctrl: 6 [1] cm; $p < 0.05$) compared to the Ctrl group. During stair descent the PN group again showed significantly higher maximum CoM-CoP separation (mean [SD] PN: 15 [3], DM: 13 [4], Ctrl: 12

[3] cm; $p < 0.05$) and significantly increased variation in CoM–CoP separation (mean [SD] PN: 8 [1], DM: 7 [1], Ctrl: 7 [1] cm; $p < 0.01$) compared to the Ctrl group. The PN group also displayed a significantly wider stance width compared to the Ctrl group during stair descent only (mean [SD] PN: 17 [3], DM: 15 [3], Ctrl: 15 [2] cm; $p < 0.05$). No differences in any variable were observed in the DM group compared to the Ctrl group during stair ascent or descent.

Conclusion: Diabetes patients with peripheral neuropathy display greater extremes in magnitude of medial-lateral sway during stair ascent and descent as well as displaying higher variability during stair ascent and descent. This indicates that patients with DPN have difficulty regulating control of balance during this challenging task. A larger and more variable medial-lateral sway means that patients with DPN are more likely to lose control of balance and experience a fall during what is known to be an activity where the risk of falls is already very high (stair walking).

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Shear forces applied to the feet during walking act for a longer duration in patients with diabetic neuropathy

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Background and aims: High plantar shear pressures are believed to play an important role in the development of diabetic foot ulcers, despite little supporting evidence. In-shoe plantar pressure systems can only measure vertical forces, whilst force platforms allow shear forces to be measured at the foot-ground interface. We investigated whether shear forces at the foot-ground/step interface are elevated in patients with diabetic peripheral neuropathy (DPN) during level ground and stair walking.

Materials and methods: Data are presented for 94 participants in three groups: patients with DPN ($n=22$), patients with diabetes but no neuropathy (DM; $n=40$) and non-diabetic controls (CTRL; $n=32$). As participants walked at a self-selected speed, ground reaction forces acting on the feet were measured from force platforms embedded into the floor (level walkway) and steps (staircase). Data were analysed using analysis of variance and post-hoc tests.

Results: Peak propulsive shear forces were no different ($P > 0.05$) between groups during stair ascent, but were lower in the DPN compared to the CTRL group during stair descent. The anterior-posterior shear force-time integrals were however, higher in the DPN compared to the CTRL group during both stair ascent (DPN: 0.30, DM: 0.26, CTRL: 0.23 N-s/kg; $P < 0.01$) and stair descent (DPN: 0.41, DM: 0.41, CTRL: 0.33 N-s/kg; $P < 0.01$). During level ground walking, peak propulsive shear forces (DPN: 1.7, DM: 2.0, CTRL: 2.1 N/kg; $P < 0.01$) and anterior-posterior shear force-time integrals (DPN: 0.56, DM: 0.61, CTRL: 0.65 N-s/kg; $P < 0.05$) were lower in the DPN compared to the CTRL group. During level ground and stair walking, the medio-lateral shear force-time integrals were significantly higher in the DPN compared to the CTRL group ($P < 0.01$).

Conclusion: Our results highlight that shear ground reaction forces during level and particularly during stair walking, are applied on the feet of patients with diabetic neuropathy for longer. This longer application of shear forces to the feet may play an important role in the development of diabetic foot ulcers. Information on the specific area of the foot affected is not known from these measurements alone and warrants further investigation linked together with in-shoe plantar pressure measures.

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Increased default mode network functional connectivity in painful diabetic neuropathy

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Background and aims: Painful diabetic peripheral neuropathy (Painful-DPN) is common and leads to considerable disability and a reduced quality of life. As there are no objective biomarkers of painful-DPN current treatments are less than optimal. Recent imaging studies using resting-state functional magnetic resonance imaging (rs-fMRI) have identified a network of brain structures that are active and connected during task-free paradigms, when the brain is at rest. This is called the default mode network (DMN). We therefore investigated the status of the DMN in painful DPN as a potential biomarker.

Materials and methods: 51 patients with type 1 diabetes (No DPN, $n=17$; Painful DPN, $n=17$ and healthy volunteers [HV], $n=17$) underwent detailed clinical and neurophysiological assessments. Rs-fMRI data were acquired at 3T (Achieva, Philips Healthcare) Blood oxygen level-dependent susceptibility-weighted dynamic datasets ($n=100$) were acquired over 225sec whilst the subject fixated on a visual cross. Data analysis was performed using independent components Analysis (ICA) with the FSL Multivariate Exploratory Linear Optimized Decomposition into Independent Components (FSL-MELODIC, FMRIB Oxford) tool and a previously validated dual-regression statistical approach.

Results: Patients with painful DPN had increased functional connectivity in the DMN areas including the cingulate gyrus, hippocampus, medial frontal cortex and precuneus, when compared to no DPN and HV subjects (TFCE, uncorrected $p < 0.001$). There was no difference in DMN functional connectivity between diabetic subjects with no DPN and HV.

Conclusion: This preliminary study suggests abnormal functional connectivity in patients with painful DPN. These findings demonstrate that chronic pain has a widespread impact on overall brain function in diabetes, and suggest that disruptions of the DMN may underlie the cognitive and behavioral impairments accompanying chronic pain.

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Abnormal neural plasticity and cortical reorganisation in underlies the painful/painless legD. Selvarajah¹, I.D. Wilkinson², E. Bolan¹, S. Tesfaye³;¹Department of Human Metabolism, University of Sheffield, ²Academic Department of Radiology, University of Sheffield, ³Academic Department of Radiology, Sheffield Teaching Hospital NHS Foundation Trust, UK.

Background and aims: The clinical paradox of chronic painful neuropathic symptoms in patients with insensate feet (the so called 'painless/painful leg') is well recognised. Using recent advances in functional magnetic resonance imaging (fMRI) we sought to phenotype the pattern of brain activation in these patients with painful diabetic neuropathy (DN) to provide new insights into this poorly understood condition. In the present study, painful stimuli of the foot and thigh were examined in painful DN subjects with and without retained foot sensation using fMRI.

Materials and methods: 26 subjects (Painless DN, n=9; Painful DN sensate, n=9; Painful DN insensate, n=8) with Type 1 diabetes and 17 healthy volunteers underwent detailed clinical and neurophysiological assessments (vibration detection thresholds, sural, common peroneal and tibial nerve conduction studies using standard procedures). Painful neuropathic symptoms were assessed using the NTSS-6 questionnaire. All Painful DN subjects had severe neuropathic pain below the knees. Before fMRI heat pain was applied to the right anterior thigh (control region) and dorsum of the foot to establish a threshold of noxious thermal stimulation capable of eliciting a response of at least 7 on an 11 point Likert scale. Painful DN insensate patients were defined as those unable to perceive a noxious thermal stimulus applied to the foot at the maximum temperature setting (39.9°C). This was repeated inside the MR scanner (Acheiva 3T, Philips Healthcare) at the predetermined threshold alternating with a pain-free baseline condition in a pseudo-randomised box-car design. Images were analysed using FSL (FEAT, FMRIB, Oxford).

Results: Thermal noxious stimulus delivered to the foot and thigh was associated with significantly increased neuronal response in both the sensory discriminatory (left somatosensory cortex [stereotactic coordinates: -12,-44,78], left thalamus [-12,-12,4] and right midbrain [8,-18,-14]; corrected $p<0.05$) and emotional affective (bilateral insular cortex [-32,18,4 and 36,18,0]; corrected $p<0.05$) and left anterior cingulate gyrus (0,28,20); corrected $p<0.05$) components of the pain matrix in Painful DN sensate subjects when compared to Painful DN insensate and Painless DN groups. However, there was abnormal response to painful foot and thigh stimulation in the somatosensory cortex that was shifted laterally to the region which generally dominates the upper limb (foot: -62,-6,26 and thigh: -60,-10,34; corrected $p<0.05$) in Painful DN insensate subjects compared with HV, Painful DN sensate and Painless DN subjects. There was also significantly increased ipsilateral somatosensory cortical (foot: 64,-6,14 and thigh: 68,-10,26; corrected $p<0.05$) activation in subjects with Painful DN insensate subjects compared to other study cohorts.

Conclusion: These results suggest a novel, dynamic reorganisation of the somatosensory (S1) representation in painful DN which explains why patients with insensate feet can have intolerable pain. A further understanding of the neural plasticity and cortical reorganisation in DN may lead to the development of more effective compounds with fewer side effects.

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OP 23 Novel regulators of insulin and glucagon secretion

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The mitochondrial transcription factor TFB2M plays a critical role in mitochondrial function and insulin secretion in pancreatic beta cellsL.M. Nicholas¹, B. Valtat¹, N.-G. Larsson², M. Abels¹, N. Wierup¹, H. Mulder¹;¹Department of Clinical Sciences, Lund University, Malmö, Sweden,²Max Planck Institute for Biology of Ageing, Cologne, Germany.

Background and aims: Insulin release from pancreatic β -cells is controlled by mitochondrial metabolism via its key role in stimulation-secretion coupling. Consequently, insulin secretion is critically dependent on mitochondrial (mt) DNA expression in these cells. Mitochondrial transcription factor B2 (TFB2M) has been newly identified to play a key role in mtDNA transcription. Its exact role in mitochondrial metabolism in β -cells, however, remains unknown. This study, therefore, investigated the role of TFB2M in mitochondrial function and insulin secretion both in vitro and in vivo.

Materials and methods: RNAi was used to knockdown (KD) Tfb2m in INS-1 832/13 cells. Mice with β -cell specific knockout of Tfb2m were generated using the Cre-loxP system. Blood glucose and insulin concentrations were measured weekly in non-fasted mice from 3 weeks of age. Mitochondrial gene expression in cells and β -Tfb2m^{-/-} islets was determined by RT-PCR. Oxygen consumption in INS-1 832/13 cells was assessed by Seahorse XF24. Mitochondrial function in 18 days β -Tfb2m^{-/-} islets was investigated by using a dye reflecting mitochondrial membrane polarization (tetramethyl-rhodamine methylester; TMRM). ATP production was determined by ATP-dependent luciferase activity in permeabilized cells. Insulin secretion from cells and isolated β -Tfb2m^{-/-} islets from 18 and 35 days old mice was determined using radioimmunoassay upon stimulation with low and high glucose concentrations as well as mitochondrial fuels. β -cell mass was determined by immunofluorescence. Data were analysed using a 1-way ANOVA with repeated measures or a Mann Whitney test. A probability level of 5% was taken to be significant.

Results: Knockdown of Tfb2m in INS-1 832/13 cells resulted in decreased expression of a number of mitochondrial encoded genes including ATP synthase protein 8, cyclooxygenase 1 and NADH dehydrogenase 1 ($P<0.001$) and 5 ($P<0.01$). These cells also had impaired basal and glucose-stimulated respiration, a reduction in ATP production and content ($P<0.01$). In line with these findings in vitro, β -Tfb2m^{-/-} mice exhibited increased plasma glucose and decreased plasma insulin concentrations ($P<0.01$) after weaning, prior to the onset of diabetes. This could be attributed to impaired insulin secretion from pancreatic islets in response to glucose and mitochondrial fuel ($P<0.05$), which was present before and persisted after weaning. Furthermore, islets from pre-weaned β -Tfb2m^{-/-} mice showed altered mitochondrial function upon glucose stimulation; the maximal decrease in TMRM fluorescence intensity from baseline was reduced compared to controls ($P<0.05$). Loss of β -Tfb2m also resulted in decreased β -cell mass and β -cell area per pancreatic area ($P<0.05$) in these mice.

Conclusion: Loss of Tfb2m in pancreatic β -cells results in mitochondrial dysfunction and consequently impaired insulin secretion in response to metabolic stimuli. These changes precede the development of clinical indicators of type 2 diabetes (T2D). Taken together, these findings indicate that TFB2M may play a pathogenic and clinically important role in the development of T2D.

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Roles of the type 2 diabetes-associated gene products Arap1 and StarD10 in the control of insulin secretionG.R.J. Carrat¹, T.J. Pullen¹, L. Marselli², G. Meur¹, P. Marchetti², G.A. Rutter¹;¹Medicine, Imperial College London, UK, ²Department of Clinical and Experimental Medicine, University of Pisa, Italy.

Background and aims: Investigation of the function of genes identified by genome-wide association studies for Type 2 diabetes (T2D) may bring a better understanding of disease aetiology and ultimately provide new therapeutic

tic targets. Recently-identified common genetic variants in the ARAP1 locus affect fasting proinsulin levels (rs11603334) and glucose-induced insulin secretion (rs1552224) in man. Here we investigate the roles of two genes implicated at this locus: ARAP1 and STAR1D10.

Materials and methods: Arap1 and StarD10 expression was silenced by siRNA and proteins overexpressed via a coding plasmid transfected by Lipofectamine 2000 (cell lines) or adenoviral infection (mouse and human islets). Total and secreted insulin were measured by radioimmunoassay.

Results: Expression quantitative trait loci (eQTL) analysis by qRT-PCR in human islets from 49 donors revealed increased expression of the short form of Arap1 (variant 1: Arap1v1) in the carriers of the risk variant at SNP rs1552224 ($p < 0.02$). Association with StarD10 mRNA levels was not detected. Over-expression of the full-length variant of Arap1 (variant 3) in MIN6 cells exerted no apparent effect on insulin secretion in response to either 30 mM glucose (control: 2.31 ± 0.28 -fold; Arap1-V3: 2.22 ± 0.25 -fold; NS) or 30 mM KCl (control: 4.24 ± 0.48 ; Arap1-V3: 4.13 ± 0.72 ; NS). However, over-expression of Arap1v1, lacking an N-terminal inhibitory domain and thus predicted to be more active, tended to inhibit insulin secretion provoked by 30 mM glucose (control: 4.01 ± 0.63 -fold; Arap1-V1: 2.84 ± 0.53 -fold; NS) or 30 mM KCl (control: 3.68 ± 0.28 ; Arap1-V1: 2.90 ± 0.32 ; $p = 0.083$). Moreover, over-expression of Arap1v1 or StarD10 in human islets reduced insulin secretion provoked by 17 mM glucose (control: 33.39 ± 9.95 -fold; Arap1V1: 19.92 ± 3.11 -fold; StarD10: 15.26 ± 1.11 -fold) and by 30 mM KCl in the case of StarD10 (control: 38.39 ± 8.46 -fold; Arap1V1: 35.22 ± 3.85 -fold; StarD10: 15.41 ± 2.04 -fold). In addition, Arap1v1 overexpression strongly reduced the total insulin content of human ($54.21 \pm 0.04\%$; $p < 0.0001$) and mouse ($50.51 \pm 0.03\%$; $p < 0.0001$) islets. Forced over-expression of StarD10 in MIN6 cells decreased insulin secretion provoked by 30 (versus 3.0) mM glucose (control: 2.54 ± 0.21 -fold; StarD10: 1.55 ± 0.10 -fold; $p < 0.01$) or 30 mM KCl (control: 5.20 ± 0.35 -fold; StarD10: 2.63 ± 0.14 -fold; $p < 0.0001$). Overexpression of StarD10 potentiated apoptosis induced by staurosporine (1 μ M) in mouse islets, assessed by TUNEL staining (control: $1.85 \pm 0.23\%$; StarD10: $4.60 \pm 0.62\%$; $p < 0.0001$). siRNA-mediated silencing of neither gene affected insulin content or secretion.

Conclusion: These data indicate that Arap1, whose expression is increased in the carriers of risk alleles at SNP rs1552224, affects islet insulin content and secretion and is thus likely to mediate the effects of these alleles on diabetes susceptibility. The demonstration that StarD10 over-expression also affects insulin secretion and beta cell survival suggests that changes in the latter gene product may also contribute. However, further eQTL studies will be required to test this hypothesis in larger cohorts.

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Diazoxide as a tool to investigate metabolic amplification of insulin secretion: a reconsideration

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Background and aims: Glucose-induced insulin secretion is mediated by a bifurcating pathway generating “triggering” and “amplifying” signals. The standard protocol to demonstrate the amplifying effect of glucose (and other nutrient secretagogues) is to depolarize the beta cells by raising the external K^+ concentration while the K_{ATP} channels are clamped open by diazoxide to ensure the generation of the Nernst equilibrium. Any further increase in secretion which is caused by the addition of glucose is then believed to be mediated by the still incompletely understood amplifying pathway. Diazoxide, however, has been reported to have sites of action in addition to the K_{ATP} channel, which may complicate the interpretation of the observed effects.

Materials and methods: The membrane potential of primary mouse islet cells was measured by the patch clamp technique using the perforated patch configuration. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with the Fura technique, the mitochondrial membrane potential with rhodamine 123 and insulin granules were visualized by insulin-EGFP. Insulin secretion was quantified by batch perfusion of islets and ELISA of the fractionated efflux. The adenine nucleotide content was measured by the luciferase method after static incubation.

Results: To depolarize the beta cell plasma membrane a K^+ concentration was used (15 mM KCl) which mimicked the slow-wave depolarization by glucose (20 mV) and raised $[Ca^{2+}]_i$ to levels comparable to those produced by 20 mM glucose. In the presence of a substimulatory glucose concentration 15 mM K^+ produced only a modest transient increase of insulin secretion. When glucose

was raised from 5 mM to 10 mM in the continuing presence of 15 mM K^+ a marked increase of insulin secretion with a biphasic pattern resulted. The addition of 250 μ M diazoxide diminished the secretion down to prestimulatory levels. The abolition of the glucose-stimulated secretion by diazoxide was unexpected since K^+ still depolarized the beta cell plasma membrane (by 18 ± 1 mV) and since the presence of 10 mM glucose was expected to ensure the generation of amplifying signals. Compared with the presence of 10 mM glucose plus 15 mM KCl the addition of diazoxide led to a slight decrease of the ATP/ADP ratio and an equally slight increase of the ATP/AMP ratio. Measuring $[Ca^{2+}]_i$ using the same protocol showed that the level established by the combined action of 15 mM K^+ and 10 mM glucose was moderately, but significantly diminished by diazoxide. Concomitantly diazoxide had a depolarizing effect on the mitochondria, which was also visible in the TIRF mode, showing that mitochondria co-exist with secretory granules in the immediate submembrane space.

Conclusion: Diazoxide does not only affect insulin secretion by the opening of K_{ATP} channels. The effect on mitochondrial function may interfere with the amplifying pathway of nutrient secretagogues, the existence of which is usually demonstrated by the use of diazoxide. The interference may not even require changes in the bulk cytosol, since mitochondria could be shown to exist in the immediate submembrane space close to secretory granules.

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Elevated expression of CaV3.1 channels impairs glucose-stimulated insulin secretion through downregulation of the exocytotic machinery

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Background and aims: Pancreatic β cells are equipped with at least seven subtypes of voltage-gated calcium (CaV) channels, including CaV1.2, CaV1.3, CaV2.1, CaV2.2, CaV2.3, CaV3.1 and CaV3.2, to conduct L-, P/Q-, N-, R- and T-type CaV currents, respectively. These CaV channels are vital to the function and survival of β cells. CaV3 channel-mediated T-type Ca^{2+} currents have long been recorded in β cells from different species including human. Enhanced T-type Ca^{2+} currents are observed in type 2 diabetic Goto-Kakizaki rat β cells and T-type Ca^{2+} currents, which are absent in normal mouse β cells, occur in nonobese diabetic mouse β cells. To date, it is not known whether the enhanced T-type Ca^{2+} currents and phenotypic switch of CaV3 channel expression in diabetic β cells are causes or consequences of the disease. The present work aimed at exploring the role of CaV3.1 channels in the pathogenesis of diabetes by elevating CaV3.1 channel expression in rat β cells.

Materials and methods: We employed multiple methods including construction of adenoviral vectors (Ad) carrying either enhanced green fluorescent protein (EGFP) or EGFP-CaV3.1, patch-clamp analysis, confocal microscopy, immunoblot assay, islet perfusion and transplantation.

Results: The present work began with construction of Ad-EGFP-CaV3.1 and Ad-EGFP. Subsequently, characterization of these constructs was performed in COS-7 cells, rat islet cells and intact islets. Ad-EGFP-CaV3.1 and Ad-EGFP effectively transduced COS-7 cells and rat islet cells. Strong EGFP signals were detected in both Ad-EGFP-CaV3.1- and Ad-EGFP-transduced cells whereas typical unitary and whole-cell T-type Ca^{2+} currents were only observed in Ad-EGFP-CaV3.1-transduced cells. These data verify that Ad-EGFP-CaV3.1 is expressed in COS-7 cells and rat islet cells resulting in enhanced T-type Ca^{2+} currents. Both Ad-EGFP-CaV3.1 and Ad-EGFP efficiently infected islet cells throughout the intact islet. Ad-EGFP-CaV3.1-infected islets released less insulin during exposure to 2.8 mM glucose. Importantly these islets displayed impaired first phase but intact second phase insulin secretion when perfused with 16.7 mM glucose. These observations demonstrate that elevated expression of CaV3.1 channels selectively impairs basal insulin release and first-phase glucose stimulated insulin secretion. More importantly, the relative abundance of synaptotagmin III, syntaxin 1A and SNAP-25 proteins in Ad-EGFP-CaV3.1-treated islets is lower than that in islets infected with Ad-EGFP. These results reveal that elevated expression of CaV3.1 channels downregulates syntaxin 1A, SNAP-25 and synaptotagmin III, which are critical for glucose-stimulated insulin secretion. Most importantly, a significant normalization of hyperglycaemia occurred in streptozotocin-induced diabetic rats transplanted with control islets or islets infected with Ad-EGFP into the anterior chamber of the eye, but not in those transplanted with islets transduced with Ad-EGFP-CaV3.1. These in vivo findings

indicate that elevated expression of CaV3.1 channels causes impaired glucose homeostasis.

Conclusion: Elevated expression of CaV3.1 channels impairs glucose-stimulated insulin secretion through downregulation of the exocytotic machinery and plays an important role in the pathogenesis of diabetes.

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NGF secretion from beta cells contributes to fine tuning of insulin secretion

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Background and aims: Islet β -cells express a range of peptides such as nerve growth factor (NGF), which was originally identified as a signaling protein regulating neuronal survival. It is now recognised that NGF is a more complex messenger involved in controlling a broad range of homeostatic responses through binding to two trans-membrane receptors, trkA and p75NTR, both expressed in islets. The role of NGF in islet function has not been established so we investigated whether β -cells possess an autocrine loop through which NGF release modulates insulin secretion.

Materials and methods: Studies were conducted with isolated mouse islets (MI) and human islets (HI), and with MIN6 pseudoislets (PI) that were generated by growing MIN6 β -cells on bacterial dishes for 10 days. NGF release from islets was determined by western blotting and quantified by ELISA, and insulin secretion was quantified by radioimmunoassay. The biological activity of β -cell-derived NGF was blocked via a NGF neutralising antibody (AbNGF) at 5 μ g/mL and trkA blockade was achieved with 5nM K252a. Immunoprecipitation studies were performed using INS-832/13 β -cells with an anti-RhoGDI antibody followed by electrophoresis and western blotting probing with anti- p75NTR.

Results: NGF expression and release from islets was identified by western blotting, and a sensitive ELISA indicated that NGF was secreted from MI in a glucose-dependent manner (2mM glucose: 17.8 ± 6.1 pg/mL; 20mM glucose 56.7 ± 2.8 pg/mL). Blockade of secreted NGF action in islets and PIs with AbNGF increased insulin output at 2mM glucose (MI $381.0 \pm 114.0\%$, $p < 0.001$; HI $150.5 \pm 17.5\%$, $p < 0.05$; PI $142.6 \pm 17.0\%$, $p > 0.2$). In contrast, HI responded to K252a treatment at 2mM glucose with an elevation in insulin secretion comparable to that seen with AbNGF blockade ($133.7 \pm 16.2\%$, $p < 0.05$), but trkA inhibition at 20mM glucose did not affect insulin release, suggesting that p75NTR is the receptor through which locally released NGF acts to inhibit GSIS from islets and pseudoislets. Consistent with this, overexpression of p75NTR in β -cells significantly inhibited GSIS (by $48.5 \pm 2.5\%$). Furthermore, immunoprecipitation studies revealed that Rho-GDI was bound to β -cell p75NTR at 2mM glucose and the complex dissociated upon glucose stimulation. Impairment of NGF biological activity with AbNGF resulted in the inability of the p75NTR/Rho-GDI complex to dissociate in response to glucose. Most likely, this affects the correct cycling of Rho-GDI small GTPases, disturbing the control on granule secretion through the actin-dependent cytoskeleton remodeling.

Conclusion: These observations support the presence of an autocrine NGF loop in islets, whose functions go beyond the canonical survival pathways: we propose that co-release of NGF with insulin following a glucose stimulus allows the fine tuning of the β -cell secretory response to prevent hypersecretion of insulin and potentially deleterious hypoglycaemia.

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Alpha-cell functionality shows gender-specific adaptations face to a nutritional insult in Wistar rats

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Background and aims: Nutritional rehabilitation after early food restriction has been extensively associated with the later appearance of metabolic disturbances, like type 2 diabetes, according to the thrifty phenotype hypothesis. This fact has been related with adaptations of beta-cell plasticity and function. Together with beta-cells, alpha-cells play a key role in the control of

glucose homeostasis and abnormalities in alpha-cell functionality have been also reported in patients with type 2 diabetes. In the present study we aimed to analyse the adaptations of alpha-cells face to a nutritional rehabilitation and gender-specific differences were investigated.

Materials and methods: The experiments were carried out in male and female Wistar rats aged of 180 days, belonging to four diet groups: control (C), fed a standard chow diet, undernourished (U), fed 35% of the daily control food intake, control high-fat (CHF) and undernourished high-fat (UHF), both fed a HFD from weaning onwards. Immunohistochemical and morphological analysis were performed to quantify alpha- and beta-cell mass, as well as the islet number and size. Functionality of alpha and beta cells was analysed in vitro by static incubation of isolated islets under different glucose concentrations (0.5, 3 and 17mM). Alpha-cell secretory capacity was studied by adding arginine (19mM) to the incubation buffer. Glucagon and insulin content were quantified in the isolated islets by RIA.

Results: Islets from HFD-fed rats showed increased size in both male and female rats, whereas islet density only increased in CHF female rats. Alpha-cell mass significantly increased in UHF rats, whereas no changes were observed in CHF rats. Both male and female CHF rats exhibited increased glucagon secretion under low glucose conditions (0.5mM) and a lack of suppression face to increasing glucose concentrations. Despite significantly increased islet glucagon content in both male and female UHF rats, gender-specific differences were observed regarding the islet functionality. While UHF male rats showed improved response to stimulating glucose concentrations, UHF female rats exhibited a nearly absence of alpha-cell response, similar to that shown by U rats. All populations showed increased glucagon secretion when analysed with 19mM arginine. However, UHF female islets showed the greater increase in glucagon secretion at 0.5mM (~4-fold) and a significant lack of suppression at 3mM (~3.6-fold). Despite the increased beta-cell mass shown by both CHF and UHF rats, a compromised beta-cell function was exhibited by these rats under stimulating glucose conditions (17mM), especially worsened in UHF female rats, showing also decreased insulin content.

Conclusion: Since glucagon release by alpha-cells is mainly regulated by glucose and a highly specific paracrine control, these results point to a defect in glucose sensitivity of alpha-cells after HFD, likely related to a deficient beta-cell-mediated paracrine control of glucagon. The worsening of these alterations in female rats evidences a gender-specific occurrence of impaired adaptations to a nutritional insult.

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OP 24 Genes and biomarkers for type 2 diabetes

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A beta cell specific protein subnetwork significantly enriched for association with GLP-1 stimulated insulin secretion: a DIRECT study

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Background and aims: The heritability of insulin secretion response (ISR) during a modified hyperglycaemic clamp with glucose, glucagon-like peptide-1 (GLP-1) and arginine stimulation has been shown to be considerable ($h^2=0.52$ to 0.80) and it is of great interest to identify genetic variants influencing these responses. Evaluating ISR with a modified hyperglycaemic clamp is not feasible in larger cohorts of sizes preferred for genome-wide association studies (GWAS). When sample sizes are small, GWAS may be complemented with systems biology approaches to aid the prioritisation of genetic variants. Within the DIRECT consortium a GWAS was performed on GLP-1 stimulated ISR and here we aimed to use data integration to add biological context to the results and facilitate variant prioritisation.

Materials and methods: GLP-1 stimulated ISR was measured with a modified hyperglycaemic clamp in 130 twins and sibs from the Netherlands twin register. The cohort was genotyped using the Illumina HumanCore Exome BeadChip and association analysis was performed using the QTassoc software and adjusted for age, sex, familial relationships and insulin sensitivity index. Gene-based P-values were mapped onto a β -cell specific protein-protein interaction (PPI) network, which was created by pruning high confidence PPIs from InWeb 3.0 using published β -cell RNAseq data. Connected components in the network enriched for high scoring genes were identified with the Cytoscape plugin jActiveModules. The significance of the top-scoring network was evaluated by converting the gene P-values to z-scores and estimating the probability of observing a combined z-score of same size or higher with 10,000 degree-preserved randomly sampled subnetworks from the β -cell PPI. Gene set enrichment analysis was performed on the top subnetwork using DAVID.

Results: None of the variants tested in the GWAS reached a genome-wide significance of $P \leq 5.0E-8$. However, the top scoring subnetwork had a significantly higher combined z-score than expected by random ($P \leq 1.0E-4$). It contained 25 genes and was most strongly enriched for the Gene Ontology terms “plasma membrane part” (Padj = $2.2E-4$), “cell junction” (Padj = $3.6E-4$) and “cell projection” (Padj = $4.9E-3$). The subnetwork contained a number of genes known to affect β -cell mass and function (FOXO1), insulin secretion (WFS1, RYR2) or to be implicated in type 2 diabetes (MAGI2, CTNNA2 and PTPRD).

Conclusion: We have identified a β -cell PPI network enriched for genes with nominal associations with GLP-1 stimulated ISR, demonstrating how data integration can highlight biological mechanisms underlying a phenotype where GWAS results on their own may be insufficient. Furthermore, the network can be used to prioritise genetic variants to take forward for replication in independent cohorts.

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FOXA2 bound sites are enriched for type 2 diabetes risk variants

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Background and aims: Risk variants for type 2 diabetes (T2D) are primarily non-coding and are enriched at regulatory features; however, specific causal variants and regulatory factors underlying risk variant activity are largely unknown. We aimed to integrate systematic regional genotyping data with genomic annotation to identify (i) regulatory factor binding sites broadly enriched for T2D risk and (ii) causal regulatory variants driving enriched signals.

Materials and methods: We used “credible sets” of SNPs that account for 99% of the probability of including the causal variant for 39 T2D association signals identified in a study of 27,116 T2D cases and 57,574 controls (MetaboChIP imputed up to 1000G). We then identified chromatin immunoprecipitation (ChIP) binding sites of 141 proteins obtained from ENCODE and several independent studies. Credible sets were tested for enrichment in the sum of posterior probabilities for variants overlapping binding sites for each factor pooled across all assayed cell types compared to variants in shuffled site locations (within 100kb). We performed enrichment tests both across all loci together and, for globally-enriched factors, at each locus individually.

Results: We identified significant enrichment for FOXA2 sites (assayed in pancreatic islets and HepG2 cells) across all risk loci ($P=3 \times 10^{-4}$). No other factor was significantly enriched. Among individual loci, there was nominally significant enrichment ($P < .05$) at 12 out of the 24 total loci containing at least one credible set variant overlapping a FOXA2 site. We identified candidate regulatory variants at 8 of these 12 loci using predictions of sequence motifs in FOXA2-bound sites. Further in silico and preliminary experimental study of allelic effects on protein binding suggests a subset are functional regulatory variants, including a variant with prior functional evidence (rs7903146 at TCF7L2).

Conclusion: These results identify potential causal variants at a set of known T2D risk loci and suggest that altered FOXA2 binding may be a common functional avenue for many T2D risk variants.

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The terminal complement complex C5b-9, but not the anaphylatoxin C5a, is cross-sectionally associated with fatty liver disease:

The CODAM study

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Background and aims: The etiological mechanisms that underlie human fatty liver disease are incompletely understood. We previously showed that certain aspects of complement activation -as reflected by systemic C3a levels- were associated with human fatty liver disease. Objective: To investigate whether activation of the terminal complement pathway -as represented by C5a and soluble (s)C5b-9- was also associated with fatty liver disease.

Materials and methods: Plasma C5a and sC5b-9 were measured in a cross-sectional evaluation of the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM, $n=508$, 61% men; 59 ± 7 yrs). As markers of fatty liver disease we used liver fat content (eLF%), estimated using a validated predictive equation, as well as liver enzymes [alanine aminotransferase (ALT), aspartate amino transferase (AST), and gamma-glutamyl transferase (GGT)], that were compiled into a standardized (std) liver enzyme (LE) score. Multiple linear regression analyses were used to investigate associations between C5a and sC5b-9 and these fatty liver disease markers. Analyses were adjusted for age, sex, impaired glucose metabolism and type 2 diabetes mellitus, cardiovascular disease, smoking, alcohol consumption, kidney function, medication, physical activity, and waist circumference. We additionally investigated whether these associations, if present, were mediated (i.e. explained) by low-grade inflammation (LGI). For this, 8 markers of LGI (TNF- α , CRP, IL6, IL8, serum amyloid A, ICAM1, ceruloplasmin and haptoglobin) were compiled into a std LGI score that was added to the fully adjusted regression models.

Results: In the fully adjusted regression models, std sC5b-9 was significantly associated with std eLF% ($\beta=0.12$ [95%CI: 0.05;0.18]) and with the std LE-score ($\beta=0.16$ [95%CI: 0.09;0.24]). In contrast, C5a was not significantly associated with either fatty liver disease marker. The association between sC5b-9 and the markers of fatty liver disease was for a substantial part (20–30%), but not fully, explained by LGI. In the final, mediated, regression model, sC5b-9 ($\beta=0.08$ [95%CI: 0.01;0.14], $\beta=0.13$ [95%CI: 0.05;0.21]) and the LGI score ($\beta=0.14$ [95%CI: 0.07;0.21], $\beta=0.14$ [95%CI: 0.06;0.23]) were both independently associated with eLF% and the LE-score, respectively. Similar results were obtained when analyses were restricted to those who consumed no-to-moderate amounts of alcohol (<30 g/d for men, <20 g/d for women, $n=400$).

Conclusion: In the CODAM cohort, plasma levels of the anaphylatoxin C5a appeared not to be associated with fatty liver disease. In contrast, a hallmark of full complement activation -i.e. formation of the (s)C5b-9 complex- was associated with human fatty liver disease, as represented by markers of hepatic fat accumulation (eLF%) and hepatocellular injury (LE-score). These associations were only partly explained by LGI and therefore suggest a po-

tential direct involvement of terminal complement activation in the aetiology of human fatty liver disease, which deserves further investigation aiming to unravel the potential mechanisms involved.

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Novel metabolite models that distinguish impaired glucose tolerance (IGT) from normal glucose tolerance (NGT)

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Background and aims: Metabolomic profiling studies have identified a number of metabolites where fasting levels are associated with dysglycemia and type 2 diabetes. The aim of this study was to identify metabolites and metabolite-based models that distinguish IGT from NGT in non-diabetic subjects.

Materials and methods: Using the stable isotope dilution technique, quantitative assays were developed for a set of 23 candidate biomarker metabolites previously linked to dysglycemia. This set included: α -hydroxybutyric acid (AHB), linoleoylglycerophosphocholine (LGPC), oleic acid, α -ketoglutaric acid, 2-aminoadipic acid, glycine, aromatic amino acids, and the 3 branched-chain amino acids and several of their catabolites. These metabolites were measured in fasting plasma samples taken just prior to an OGTT from 1,679 non-diabetic subjects: 979 from the RISC Study 3 year follow up (11.5% have IGT) and 679 subjects from the DMVhi cohort in the DEXLIFE project (11.8% have IGT).

Results: Random forest decision tree analysis using subject metabolite, anthropometric, and metabolic characteristic data were generated to rank variables for their ability to distinguish IGT from NGT. The top 4 were found to be AHB, fasting glucose, LGPC, and oleic acid in RISC and fasting glucose, age, LGPC, and AHB in DMVhi. Multivariate models for estimating risk of IGT were evaluated in both cohorts using AUCs calculated from the corresponding ROC curves. AUCs generally did not increase in models having more than 7 variables. A number of 7 variable, metabolite only models were developed that had AUCs >0.80. For example, in RISC, a model consisting of AHB, glucose, LGPC, oleic acid, glycine, creatine, and 2-oxoisoleucine had an AUC of 0.825. In contrast, a model consisting of age, sex, BMI, glucose, insulin and HDL had a significantly lower ($p=0.0001$) AUC of 0.731.

Conclusion: AHB and LGPC have been identified as metabolite markers of IGT and metabolite-based models may be useful for identifying IGT in non-diabetic subjects

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Causal effect of decreased LDL cholesterol and increased blood pressure on higher incidence of type 2 diabetes by Mendelian randomisation in the Malmö Diet and Cancer Study

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Background and aims: Obesity, dyslipidemia and hypertension associate with type 2 diabetes (T2D). However, the relationship between these traits and T2D may not be causal due to confounding and reverse causation. In the Mendelian randomization approach genetic variants are assumed to be randomly distributed, and can be used as instrumental variables to estimate causal relationships. The aim of this study was to investigate if BMI, systolic blood pressure (SBP) and lipid traits are causally associated with T2D incidence by using genetic risk scores (GRSs) of single nucleotide polymorphisms (SNPs) identified in genome wide association studies (GWAS) for these traits as instrumental variables.

Materials and methods: In total, 27254 non-diabetic participants (61% females) from the population based Malmö Diet and Cancer Study (MDCS), collected at baseline during 1990–1996 (age 58 ± 8 years, BMI 26 ± 4 kg/m²), were genotyped for SNPs associating with BMI ($N=31$), high density lipoprotein cholesterol (HDL, $N=41$), low density lipoprotein cholesterol (LDL, $N=32$), triglycerides (TG, $N=26$) and SBP ($N=29$). Trait-specific weighted GRSs were created using PLINK software. During a mean follow-up time of

15 ± 4 years, 3248 incident T2D and 3977 cardiovascular disease (CVD) cases were identified. At baseline, BMI and SBP were measured for all individuals and fasting blood lipid levels were analyzed in a random sub-cohort (MDC-CC) of 5284 individuals of which 781 developed T2D and 754 CVD. COX-regression was used to analyze the association between BMI, SBP, HDL, LDL and TG, and incidence of T2D per standard deviation (SD). For Mendelian randomization, a two-stage least square regression method was used with the GRSs as instrumental variables. The predicted values for the first stage of regression of the lipid traits on their respective GRSs were estimated from MDC-CC for all MDCS. Adjustments were made for age, sex, lipid-lowering and antihypertensive medication.

Results: As expected, baseline levels of higher BMI, SBP, LDL and TG, and lower HDL associated with higher incidence of T2D (HR 1.83 [1.78–1.88], $P<0.00001$; 1.34 [1.29–1.38], $P=3 \times 10^{-59}$; 1.17 [1.08–1.25], $P=0.0004$; 1.23 [1.20–1.27], $P=2 \times 10^{-50}$ and 1.66 [1.51–1.82], $P=4 \times 10^{-27}$, respectively). The instrumental variable analysis revealed causal effect of increased BMI and SBP but decreased LDL on higher incidence of T2D (HR 1.92 [1.30–2.83], $P=0.001$; 1.99 [1.14–3.48], $P=0.02$ and 1.14 [1.00–1.28], $P=0.04$, respectively). The instrumental variable analysis did not indicate causality between HDL or TG and incidence of T2D (0.90 [0.78–1.03], $P=0.13$ and 1.07 [0.91–1.26], $P=0.42$, respectively). The instrumental variable analysis indicated a causal effect of increased SBP, LDL and TG on higher incidence of CVD (HR 1.73 [1.04–2.88], $P=0.03$; 1.21 [1.08–1.36], $P=0.001$ and 1.17 [1.01–1.36], $P=0.04$, respectively).

Conclusion: In this large prospective study the Mendelian randomization analysis indicated causal effects of increased BMI and SBP and decreased LDL on higher incidence of T2D. Our results suggest opposite causal effects of LDL on the risk of T2D and CVD, and raise the question about the potential deleterious effects of LDL lowering agents on the risk of T2D. Further, our study emphasizes the importance of weight loss and blood pressure control in prevention of T2D.

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Genome wide meta-analysis highlights the role of genetic variation in RARRES2 in regulation of circulating serum chemerin

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Background and aims: Chemerin is an adipokine proposed to link obesity and chronic inflammation of adipose tissue. Genetic factors determining chemerin release from adipose tissue are yet unknown.

Materials and methods: We conducted a meta-analysis of genome-wide association studies GWAS for serum chemerin in three independent cohorts from Europe (Sorbs and KORA from Germany and PPP-Botnia study from Sweden; total $N=2,791$). Furthermore, we measured mRNA expression of genes within the associated loci in lymphocytes and adipose tissue by quantitative RT-PCR and performed mRNA expression-genotype association studies.

Results: Heritability of circulating chemerin levels was 16.2%. Thirty one single nucleotide polymorphisms (SNPs) at chromosome 7 within the *RARRES2* locus reached genome-wide significance ($p<10^{-7}$) in the meta-analysis (strongest evidence for association at rs7806429 with $p=7.8 \times 10^{-14}$, $\beta=-0.067$). All other SNPs within the cluster were in strong linkage disequilibrium with rs7806429 ($r^2 > 0.43$ in the Sorbs). The results in the subgroup analyses in males and females were consistent with the results in the total cohort. Although effect sizes for all genome-wide significant SNPs were

stronger in females than in males, no SNP gender interaction was observed. Furthermore, rs7806429 was associated with mRNA expression of *RARRES2* in visceral adipose tissue in women ($p < 0.05$ after adjusting for age, sex and body mass index).

Conclusion: The present meta-analysis of GWAS combined with mRNA expression studies highlights the role of genetic variation in *RARRES2* in the regulation of circulating chemerin concentrations.

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OP 25 Novel insulin formulations and combinations

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The effect of insulin degludec in combination with liraglutide and metformin in patients with type 2 diabetes requiring treatment intensification

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Background and aims: Several studies have evaluated the combination of a basal insulin and a glucagon-like peptide-1 (GLP-1) analogue in addressing the multiple concomitant defects in glucose homeostasis seen in type 2 diabetes (T2D). The aim of this trial was to investigate the efficacy of insulin degludec (IDeg) compared with placebo in improving glucose control in patients with T2D, treated with liraglutide (lira) and metformin and qualifying for treatment intensification.

Materials and methods: In this 26-week, double-blind trial, patients with T2D who remained inadequately controlled (HbA_{1c} 7–9%, inclusive) after a 15-week run-in period with initiation and intensification of liraglutide to 1.8 mg in combination with metformin (≥ 1500 mg) were randomised (1:1) to addition of once-daily IDeg ($n=174$) or placebo ($n=172$), with dosing of both IDeg and placebo based on titration guidelines (fasting plasma glucose [FPG] target 4.0–5.0 mmol/L).

Results: Patient characteristics were generally similar at randomisation: mean HbA_{1c} was 7.5% (IDeg+lira) vs. 7.6% (placebo+lira), BMI 32.0 kg/m² vs. 32.4 kg/m², duration of diabetes 9.7 years vs. 9.3 years and FPG 8.7 mmol/L vs. 9.1 mmol/L. At 26 weeks, IDeg+lira was superior to placebo+lira in improving glycaemic control in terms of HbA_{1c} (estimated treatment difference [ETD]: -0.92% [-1.10; -0.75]_{95%CI}, $p < 0.0001$) with observed mean reductions of 1.04% and 0.16%, respectively. IDeg+lira was more effective in lowering FPG than placebo+lira, ETD -2.55 mmol/L [-3.07; -2.02]_{95%CI}, $p < 0.0001$ with observed mean reductions of 2.60 mmol/L and 0.28 mmol/L, respectively. At 26 weeks, IDeg dose was 51 U while placebo was titrated to a dose corresponding to 105 U. During lira run-in, mean body weight in those later randomised to trial treatment decreased from 95.4 kg to 92.3 kg. After 26 weeks of treatment, patients randomised to IDeg+lira had gained on average 2.0 kg, while patients randomised to placebo+lira had an additional weight reduction of 1.3 kg during treatment. Rates (episodes per exposure-year) of confirmed hypoglycaemia were low in both groups, higher with IDeg+lira than with placebo+lira (0.57 vs. 0.12), estimated treatment ratio: 4.67 [2.07; 10.56]_{95%CI}, $p = 0.0002$. Rates of nocturnal confirmed hypoglycaemic episodes were low and similar for both groups (0.05 and 0.03, respectively, $p = \text{NS}$). There were no episodes of severe hypoglycaemia in either treatment arm. No safety issues were identified with IDeg+lira with respect to adverse events or standard safety parameters.

Conclusion: For patients with T2D treated with lira+metformin and qualifying for treatment intensification, addition of IDeg compared with placebo significantly improved glycaemic control with low rates of overall hypoglycaemia (higher with IDeg than placebo) and similar rates of nocturnal hypoglycaemia compared with placebo.

Clinical Trial Registration Number: NCT01664247

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Similar efficacy and safety with LY2963016 insulin glargine compared with insulin glargine in patients with type 1 diabetes mellitus: the ELEMENT 1 studyR.K. Pollom¹, T. Bevins², D. Dahl³, J. Rosenstock⁴, W.J. Huster¹, L.L. Ilag¹, M.J. Prince¹¹Eli Lilly and Company, Indianapolis, ²Texas Diabetes & Endocrinology, Austin, USA, ³Gemeinschaftspraxis für Innere Medizin und Diabetologie, Hamburg, Germany, ⁴Dallas Diabetes and Endocrine Center, USA.

Background and aims: LY2963016 (LY IGLar) and insulin glargine (Sanofi-Aventis; IGLar) are both insulin glargine products, with identical amino acid sequences. Even with identical primary structure, protein-based therapeutics manufactured by distinct processes must be shown to be clinically similar.

Materials and methods: A 52-week, Phase 3, randomized, open-label, parallel study, with a 24-week treatment period and a 28-week extension, was undertaken to compare the efficacy and safety of LY IGLar once daily (QD) vs IGLar QD in combination with pre-meal insulin lispro thrice daily in patients with T1DM ($HbA_{1c} \leq 11.0\%$). LY IGLar and IGLar were administered with prefilled pen injectors. The primary aim was to test the non-inferiority (0.3% margin) of LY IGLar to IGLar as measured by change in HbA_{1c} from baseline to 24 weeks. Testing for non-inferiority of IGLar to LY IGLar was also performed and pre-specified as a complementary hypothesis, which if met along with the primary aim, would demonstrate equivalent efficacy between LY IGLar and IGLar. Insulin doses were adjusted during the study to achieve glycemic targets ($HbA_{1c} < 7\%$, fasting plasma-equivalent glucose ≤ 6.0 mmol/L). Safety assessments included hypoglycaemia, adverse events (AEs) and antibody responses.

Results: Most patients (LY IGLar, 81.3%; IGLar 87.6%) were receiving IGLar prior to randomization. Both treatment groups had within-group statistically significant ($p < .001$) decreases in mean HbA_{1c} values from baseline. The change in HbA_{1c} from baseline with LY IGLar was non-inferior to IGLar (Table). Non-inferiority of IGLar to LY IGLar was also demonstrated; thus, the criteria for equivalence in clinical efficacy between LY IGLar and IGLar were met. At 24 and 52 weeks, there were no treatment differences in secondary efficacy or safety outcomes including hypoglycaemia and treatment-emergent antibody response (Table). At 52 weeks, similar findings were seen between LY IGLar and IGLar in mean [SD] overall rates (events/patient/year) of nocturnal hypoglycaemia (LY IGLar, 16.1 [20.2]; IGLar, 17.3 [19.5], $p = .250$) and severe hypoglycaemia (LY IGLar, 0.1 [0.5]; IGLar, 0.1 [0.5], $p = .826$), and in mean [SD] weight gain (kg) (LY IGLar, 0.9 [3.3]; IGLar, 0.6 [3.7], $p = .253$). AE frequency was the same with LY IGLar (62%) and IGLar (62%) ($p > .999$).

Conclusion: LY IGLar compared with IGLar, used in combination with insulin lispro, provided equivalent efficacy and a similar safety profile in patients with T1DM.

Table

Outcome Measure LSM (SE) Unless Otherwise Indicated	LY IGLar 24 Weeks N=268 ^a	IGlar 24 Weeks N=267 ^a	p-value	LY IGLar 52 Weeks N=268 ^a	IGlar 52 Weeks N=267 ^a	p-value
HbA_{1c} (%)						
Change from Baseline	-0.352 (0.053)	-0.480 (0.054)	.065	-0.256 (0.057)	-0.278 (0.058)	.737
LSM Diff [95% CI] (LOCF)	0.108 [-0.002, 0.219]			0.020 [-0.096, 0.140]		
N (%) of Patients reaching $HbA_{1c} < 7.0\%$ (LOCF)	92 (35)	86 (32)	.646	81 (30)	67 (25)	.209
FBG by SMBG (mmol/L)						
Change from Baseline	-0.55 (0.25)	-0.52 (0.25)	.912	-0.50 (0.27)	-0.04 (0.27)	.101
LSM Diff [95% CI] (LOCF)	-0.03 [-0.55, 0.49]			-0.46 [-1.01, 0.09]		
Daily Mean Blood Glucose (LOCF), mmol/L	8.32 (0.13)	8.31 (0.13)	.960	8.29 (0.13)	8.51 (0.14)	.128
Basal/Prandial Insulin Dose, U/kg/day (LOCF), LSM	0.370/35	0.360/35	.235/ .726	0.380/37	0.360/37	.159/ .967
Total Hypoglycaemia ^b Rate (events/patient/year)	86.5 (77.3)	89.2 (80.1)	.717	77.0 (68.7)	79.8 (74.5)	.738
Mean (SD), Overall	25 (9.4)	17 (6.4)	.202	29 (10.9)	25 (9.4)	.569
N (%) of Patients with TEAR, Overall						

^aFull Analysis Set; N numbers reflect maximum sample size^bIncluding events with blood glucose ≤ 3.9 mmol/L, if blood glucose was available
LOCF = last observation carried forward (endpoint); LSM = least squares mean; TEAR = treatment emergent antibody response (including patients who were antibody negative at baseline and developed antibody binding values $\geq 1.26\%$ postbaseline, or patients with detectable antibody levels at baseline with at least a 1% increase in antibody binding value and which is 30% greater than baseline)

Clinical Trial Registration Number: NCT01421147

Supported by: Eli Lilly and Company

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Treatment intensification with IDegAsp BID vs IDeg OD plus IAsp in insulin-treated patients with type 2 diabetes: a randomised, controlled phase 3 trialJ.G. Cooper¹, T.R. Pieber², B. Cariou³, L. Endahl⁴, J. Zacho⁴, H.W. Rodbard⁵¹Stavanger University Hospital, Norway, ²Medical University of Graz, Austria, ³L'Institut du Thorax, Nantes University Hospital, France,⁴Novo Nordisk A/S, Søborg, Denmark, ⁵Endocrine and Metabolic Consultants, Rockville, USA.

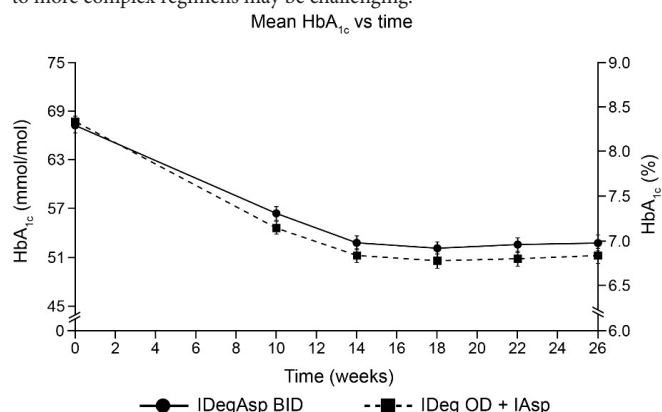
Background and aims: This study compared the efficacy and safety of two regimens in the management of adults with TD2M previously treated with

basal insulin and in need of insulin intensification to improve glycaemic control in the postprandial period: (1) IDegAsp (a fixed and soluble combination of 70% insulin degludec IDeg and 30% insulin aspart (IAsp), administered twice daily (BID), and (2) a basal-bolus regimen with once daily IDeg (OD) with IAsp 2–4 times daily.

Materials and methods: In this 26-week randomised multinational phase 3b trial, participants (mean: age 60 years, HbA_{1c} 8.3%, duration of diabetes 13 years) previously treated with a basal insulin (insulin glargine [64%]) were randomised (1:1) to IDegAsp BID ($n=138$) or IDeg OD plus IAsp 2–4 times daily ($n=136$). IDegAsp was administered with the two main meals of the day.

Results: HbA_{1c} was reduced with IDegAsp BID and IDeg OD plus IAsp regimens (to 7.0 and 6.8%, respectively; see Figure), with no significant difference between the two treatments. IDegAsp did not achieve non-inferiority, as the 95% CI exceeded the pre-defined non-inferiority limit (Estimated Treatment Difference [ETD] 0.18, 95% CI -0.04; 0.41). A significantly lower total daily insulin dose of 1.11 U/kg for IDegAsp BID vs 1.34 U/kg for IDeg OD plus IAsp (estimated ratio 0.88, 95% CI 0.78; 1.00, $p < 0.05$) was observed. Change in body weight (LOCF analysis) at end of trial was +2.75 kg and +3.76 kg for IDegAsp BID and IDeg OD plus IAsp (ETD -1.04, 95% CI -1.99; -0.10, $p < 0.05$). Fewer confirmed hypoglycaemia episodes (self-reported plasma glucose < 3.1 mmol/L) were reported for IDegAsp BID vs IDeg OD plus IAsp (11.6 vs 13.6 events/patient year exposure [Relative Rate (RR) 0.81, 95% CI 0.61; 1.07, $p = NS$]). Frequency of nocturnal confirmed hypoglycaemia episodes (onset 00.01–05.59 h) was lower with IDegAsp BID vs IDeg OD plus IAsp (1.23 vs 1.55 events/patient year exposure, RR 0.80, 95% CI 0.50; 1.29, $p = NS$).

Conclusion: HbA_{1c} was reduced with IDegAsp BID and IDeg OD plus IAsp, with no significant difference between the regimens. The 95% confidence interval for the HbA_{1c} treatment difference did cross the pre-specified non-inferiority margin for the primary analysis (however, all pre-specified sensitivity analyses did achieve non-inferiority). IDegAsp BID was associated with significantly lower total daily insulin doses and less weight gain, with non-significant lower rates of confirmed and nocturnal confirmed hypoglycaemia episodes compared with IDeg OD plus IAsp. Thus, IDegAsp BID offers the potential for a simple alternative to basal bolus treatment in patients who require intensification of basal insulin regimens, especially where adherence to more complex regimens may be challenging.



Clinical Trial Registration Number: NCT01713530

Supported by: Novo Nordisk A/S

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Glycaemic control and hypoglycaemia with new insulin glargine 300 U/mL in people with type 1 diabetes (EDITION 4)P.D. Home¹, R.M. Bergenstal², M.C. Riddle³, M. Ziemien⁴, M. Rojeski⁵, M. Espinas⁶, G.B. Bolli⁷¹Newcastle University, Newcastle upon Tyne, UK, ²International Diabetes Center at Park Nicollet, Minneapolis, ³Oregon Health & Science University, Portland, USA, ⁴Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany, ⁵Sanofi, Bridgewater, USA, ⁶Sanofi, Paris, France,⁷University of Perugia, Italy.

Background and aims: EDITION 4 studied the efficacy and safety of new insulin glargine (300 U/ml; Gla-300) compared with glargine 100 U/ml (Gla-100) in people with type 1 diabetes mellitus.

Materials and methods: In this 6-month, multinational, multicentre, open-label study, participants ($n=549$, BMI 27.6 kg/m², diabetes duration 21.0

years, HbA_{1c} 8.1 % [65 mmol/mol]) were randomised 1:1:1 to once-daily Gla-300 or Gla-100, morning or evening, while continuing mealtime insulin. **Results:** Overall, Gla-300 was non-inferior to Gla-100 for HbA_{1c} change from baseline (primary endpoint) (LS mean change [SE] -0.40 [0.05] % (-4.4 [0.6] mmol/mol) and -0.44 [0.05] % (-4.8 [0.6] mmol/mol); LS mean difference 0.04 [95% CI: -0.10 to 0.19] % (0.4 [-1.1 to 2.1] mmol/mol)). Event rates of confirmed (≤ 3.9 mmol/L [≤ 70 mg/dl]) or severe hypoglycaemia at any time of day (24 h) were similar for the two groups, while nocturnal hypoglycaemia was lower in the Gla-300 group compared with the Gla-100 group during the first 8 weeks of the study (Table). Severe hypoglycaemia was observed in 6.6% (Gla-300) and 9.5% (Gla-100) of participants. Neither glycaemic control nor hypoglycaemia differed between insulins or times for morning and evening injection groups. Total insulin dose increased to a somewhat greater extent for Gla-300 compared with Gla-100 (change from baseline +0.19 versus +0.10 U/kg). Weight gain was statistically significantly lower with Gla-300 versus Gla-100 (LS mean difference -0.56 [95% CI: -1.09 to -0.03] kg, $p=0.037$). There was no difference in adverse events between the two groups. **Conclusion:** In conclusion, new insulin glargine 300 U/ml provided comparable glycaemic control versus glargine 100 U/ml, while nocturnal hypoglycaemia was less frequent during the first 8 weeks of treatment.

Table – Hypoglycaemic events per participant-year in the two insulin groups

	Gla-300 (N=274)	Gla-100 (N=275)	RR (95% CI)
Total participant-years	124.1	126.8	
Nocturnal (00:00–05:59 h) confirmed (≤ 3.9 mmol/L [≤ 70 mg/dl]) or severe			
Baseline to month 6	8.0	8.9	0.90 (0.71 to 1.14)
Baseline to week 8	7.8	11.2	0.69 (0.53 to 0.91)
Week 9 to month 6	8.1	7.9	1.04 (0.80 to 1.36)
Any-time (24 h) confirmed (≤ 3.9 mmol/L [≤ 70 mg/dl]) or severe			
Baseline to month 6	78.4	72.5	1.09 (0.94 to 1.25)
Baseline to week 8	87.4	89.5	0.98 (0.85 to 1.13)
Week 9 to month 6	73.9	64.2	1.16 (0.98 to 1.37)

CI, confidence interval; RR, rate ratio.

Clinical Trial Registration Number: NCT01683266

Supported by: Sanofi

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Recombinant human hyaluronidase pretreatment of CSII cannula sites provides comparable glycaemic control with reduced hypoglycaemia in T1DM compared to usual CSII

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Background and aims: Recombinant human hyaluronidase (rHuPH20) is FDA-approved to increase dispersion and absorption of injected or infused drugs. In CSII, a single pretreatment of the cannula site with rHuPH20 accelerates exposure to and action of bolus doses of rapid analogs for up to 3 days of catheter use. This study was performed to characterize the clinical attributes of this treatment in an outpatient cohort of T1DM.

Materials and methods: 456 subjects with T1DM (age 48±13 years, BMI 28.5±5.1, screening A1C 7.8±0.7) were randomized 3:1 to CSII with rHuPH20 pretreatment or usual CSII for 6 months.

Results: A1C fell 0.14% from 7.69% with rHuPH20 and 0.18% from baseline 7.70% for CSII alone. The primary endpoint of A1C noninferiority (0.4% margin) was achieved with a treatment difference of 0.05% (95% CI -0.08 to 0.18) with similar % of subjects reaching A1C <7.0% (20.9% with rHuPH20 and 17.5% for CSII alone, $p=.45$). Mean overall 90 min post-meal glucose excursion was 1.04 mmol/L with rHuPH20 and 1.09 mmol/L for CSII alone ($p=.79$). There were fewer hypoglycemic events (HEs) with rHuPH20 than for CSII alone. The protocol specified primary HE analysis was based on SMBG-based event rates after a month of active titration following randomization; these data were derived from 261,341 individual SMBG readings. Hypoglycemia results are summarized in the table. Adverse event rates were generally comparable between treatments, although rHuPH20 pretreatment

was associated with an increased incidence of infusion site pain (typically mild transient burning) from 6.2% to 14.9%.

Conclusion: We conclude that pretreatment of CSII cannulas with rHuPH20 is well tolerated and results in similar glycemic control in T1DM with reduction of hypoglycaemia.

	rHuPH20 pretreatment	Usual CSII	Difference (p-value)
A1C (Change from Baseline)	-0.14%	-0.18%	0.05% ($p=.45$)
Overall 90 min post meal excursion (mmol/L)	1.04	1.09	-0.04 ($p=.79$)
Hypoglycemia (events/subject-month)			Rate Ratio (p-value)
≤ 3.9 mmol/L	12.05	13.75	0.88 ($p=.08$)
<3.1 mmol/L	3.10	4.01	0.77 ($p=.02$)
Nocturnal (≤ 3.9 mmol/L)	1.70	2.14	0.79 ($p=.02$)
Severe	0.0061	0.0157	0.39 ($p=.08$)

Clinical Trial Registration Number: NCT01848990

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A novel concentrated recombinant human insulin formulation with improved ultra-rapid action for continuous subcutaneous infusion therapy

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Background and aims: BIOD-531, a U-400 formulation of recombinant human insulin, EDTA, citrate and MgSO₄, is associated with an accelerated onset of action compared to Regular Human Insulin U-500 (U-500R) and Insulin Lispro Mix75/25, and has a basal duration profile in obese non-diabetic subjects and in diabetic swine. In order to assess the potential of BIOD-531 for open and closed-loop continuous subcutaneous insulin infusion (CSII) therapy, we evaluated the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of BIOD-531 vs. U-500R and Insulin Lispro U-100 (Humalog, IL) following pump bolus administration and compared it to the timing of subcutaneous (sc) injection in miniature diabetic swine.

Materials and methods: Test formulations consisting of IL, U-500R, BIOD-531 or BIOD-530 (BIOD-531 without MgSO₄) were administered using a crossover design by either Animas pump (Studies 1 and 2) or sc injection (Studies 3 and 4). On the morning of each study, miniature diabetic swine were given a sc dose (0.25 U/kg) of test formulation, followed by a meal. Blood glucose and plasma insulin were sampled from -30 to 480 min post dose. Plasma insulin was measured by an ELISA method and glucose concentration was determined by YSI. Data from each study were compared for significant differences from BIOD-531 using Students t-test.

Results: Key PK timing parameters ($T_{50\%max}$, T_{max} and $AUC_{0-20min}$) and onset of action PD parameter T-BG_{50%Minimum} (time to 50% glucose nadir) are shown in Table below. BG_{Minimum} is the lowest baseline subtracted glucose value or nadir. The rate of absorption and onset of action of BIOD-531 are not significantly different from IL and faster than U-500R following pump bolus and sc administration. In addition, the PK and PD profiles comparing BIOD-531 vs. IL are similar following pump bolus or sc injection.

Conclusion: These data suggest the potential utility of BIOD-531 as CSII therapy for insulin resistant type 2 diabetes patients who require high doses of insulin. Furthermore, BIOD-531 may be useful to help conserve space in future Artificial Pancreas systems.

Pharmacokinetic and Pharmacodynamic Profiles of BIOD-531/530 ^a Vs. IL U-100 or U-500R Following Pump Bolus Administration or Subcutaneous Injection in Miniature Diabetic Swine									
Pharmacokinetic (PK) or Pharmacodynamic (PD) Parameter		Pump Bolus Administration				Subcutaneous Injection			
		Study 1		Study 2		Study 3		Study 4	
		BIOD-531	U-500R	BIOD-531	IL	BIOD-531	IL	BIOD-530 ^b	U-500R
PK	T _{50%max} (min)	12±4	26±7	13±3	17±3	11±2	21±4*	11±2	27±5*
	T _{max} (min)	31±9	74±17	73±19	29±5	76±18	55±8	42±12	94±17*
	AUC _{0-20min} (μU/mL*min)	863±86	581±125*	758±78	961±285	1081±182	904±245	1409±151	720±154*
	C _{max} (μU/mL)	93±14	126±28	96±10	129±17	125±17	153±16	155±16	135±23
PD	T-BG _{50%Minimum} (min)	40±23	71±24	20±5	30±7	17±2	28±4	24±2	83±9
	BG _{Minimum} (mg/dL)	-245±27	-244±26	-208±34	-250±36	-244±25	-244±17	-194±5	-178±7

^aBIOD-530 is BIOD-531 without MgSO₄. ^bSignificant difference from BIOD-531/530 at p<0.05

^aBIOD-530 is BIOD-531 without MgSO₄. ^bSignificant difference from BIOD-531/530 at $p<0.05$

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OP 26 Pregnancy and diabetes

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Pregestational diabetes (type 1 and 2), gestational diabetes: data from the French population in 2011

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Background and aims: Pregestational diabetes increases the risks of congenital malformations and perinatal complications. The risks incurred by newborn infants in the case of gestational diabetes (GD) are debated. There are no national data available in France except for data from multicenter cohorts. We evaluated the risks of complications according to the type of maternal diabetes from the French birth cohort in 2011.

Materials and methods: Data were obtained from the PMSI (French hospital discharge database) and the SNIIRAM (French national health insurance information system). All childbirths and terminations of pregnancy (TOP) after 22 weeks of gestation, due to medical reasons were selected. The mother's diabetes was identified by an algorithm based on the consumption of antidiabetics and hospitalization diagnoses before and during pregnancy. An identifier in the PMSI links mothers and infants, thus enabling analyses of associations between the mother's diabetes and outcome.

Results: 806 579 childbirths / TOP > 22 weeks were identified in the PMSI. The Mother - infant chaining was obtained for 474 614 births in public institutions. 1257 (0.16%) type 1 diabetes (T1D), 1896 (0.24%) type 2 diabetes (T2D) and 51 701 (6.4%) GD were identified, with a mean age respectively of 30.3, 33.3 and 32 years. In the case of T1D and T2D, the risks were respectively increased for the following complications (OR adjusted on mother's age [95%CI]): preterm birth (gestational age < 38 weeks) (6.6 [5.9-7.4] and 3.7 [3.3-3.9]), caesarean section (4.3 [3.8-4.8] and 2.9 [2.7-3.2]), preeclampsia and eclampsia (6.7 [5.6-8.2] and 3.9 [3.2-4.7]), macrosomia (birth weight (BW) > 90th percentile) (7.0 [6.1-8.0] and 3.9 [3.4-4.4]) perinatal death (2.2 [1.4-3.4] and 3.0 [2.2-4.1]), perinatal asphyxia (3.3 [2.2-5.1] and 2.5 [1.6-3.7]), respiratory distress syndrome (OR adjusted on mother's age and gestational age): 2.6 [2.0-3.4] and 1.9 [1.5-2.5]), brachial plexus trauma and/or collarbone fractures in cases of vaginal delivery (8.5 [4.9-14.8] and 2.9 [1.5-5.9]), cardiac malformations (4.4 [3.0-6.5] and 3.2 [2.2-4.7]). Data on macrosomia are respectively for DT1 and DT2: BW > 4 kg 16.7% and 13.4%; BW > 90th percentile 42.5 % (n=354) and 30.4% (n=348). In the case of GD, the risk of certain outcomes was lower: prematurity (1.35 [1.32-1.38]), caesarean section (1.46 [1.44-1.49]), preeclampsia and eclampsia (1.55 [1.46-1.65]), macrosomia (BW > 90th percentile) (1.7 [1.6-1.8]) respiratory distress syndrome (1.2 [1.1-1.3]). The risks were not increased for the others complications compared to the population without diabetes. Concerning macrosomia, 9.0% of the newborn had a BW > 4 kg in GD, and 6.6% in the absence of diabetes. 15.7% of the newborn had a BW > 90th percentile in GD and 9.4% in the absence of diabetes.

Conclusion: The rate of perinatal complications remains high for both types of pregestational diabetes, especially T1D. The link between gestational diabetes and macrosomia is confirmed. Some complications associated with gestational diabetes are slightly increased (caesarean section, prematurity, eclampsia, respiratory distress syndrome), others are not (brachial plexus trauma and/or collarbone fractures, death, perinatal asphyxia).

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Maternity care at a superspecialised unit for women with type 1 diabetes results in pregnancy outcome comparable to background population

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Background and aims: Pregnancies in women with Type 1 diabetes are more often complicated by preeclampsia, prematurity, caesarean section, malformation, macrosomia, stillbirth and postnatal hypoglycaemia in the infant. The rate of complications is mainly depending on the blood glucose regulation. Our model for pregnant women with Type 1 is based on a specialised maternity unit including obstetrician, diabetologist, midwife and dietician. Women are required to take seven self monitored glucose measurements

daily, all values are sent electronically to the hospital and followed up weekly by a phone call from a diabetologist. The insulin doses are adjusted frequently to optimize glucose levels. An extra ultrasound is done late in pregnancy and if growth is rapid there is a liberality to induce labour. This study is aimed to compare outcome of pregnancy in women with Type 1 diabetes with the Swedish background women and also to evaluate if higher insulin dose could further improve outcome.

Materials and methods: All consecutive pregnant women with Type 1 diabetes who delivered infants during 2000-2013 (n=266) at our hospital were compared to all women in Sweden who delivered infants during the same period. Data from background population was obtained from the Swedish Medical Birth Register, which has a very high rate of ascertainment. In the next step we compared outcome in pregnancies in Type 1 diabetic women who delivered in 2000-2006 (n=1418) with those who delivered in 2007-2013 (n=148). For women in the later time period we tried to further increase insulin doses to improve metabolic regulation.

Results: For women with Type 1 diabetes birth weight was only 59 g higher (3566 compared to 3507), despite a shorter gestational length (37.7 vs. 39.3 weeks; p=0.001) and higher frequency of sectio (44% vs. 17%; p<0.001). The frequencies of preeclampsia, instrumental deliveries, postnatal hypoglycaemia, intensive care of infant, malformations and stillbirth were not significantly different. Women with partus 2000-06 compared to 2007-13 had equal duration of diabetes (15.5 yrs; p=ns), weighted same (69.4 vs. 71.3 kg; p=ns), body length were same (167; p=ns), they gained equal in weight during pregnancy (14.7 vs. 15.2 kg; p=ns), had equal insulin dose at start (49.7 vs. 50.6 IU; p=ns), same HbA1c at start (56 vs. 58 mmol/mol; p=) and gestational length was equal (264 vs. 265 days; p=ns). The women in the later group were treated with higher doses of insulin at the end of pregnancy (79.4 vs. 96.3 IU; p=0.001), but despite this, HbA1c were lower in the first group (40 vs. 42 mmol/mol; p=0.03) and birth weights did not differ (3619 vs. 3565 g; p=0.6). There were no differences in the frequencies of sectio (42% vs. 38%; p=ns), prematurity (19% vs. 22%; p=ns), malformations of all types (7.6% vs. 6.1%; p=ns), Apgar <7 at 5min (11.2 vs. 8.8%; p=ns), frequency of hypoglycaemia (24% vs. 19%; p=ns) or need of postnatal intensive care (47% vs. 35%; p=ns).

Conclusion: Pregnancy outcome in women with Type 1 diabetes was very close to results in the Swedish population. Further increase in insulin dose did not improve the results. This superspecialised maternity care for women with diabetes enables an almost normal outcome of pregnancy.

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Screening for diabetic retinopathy in pregnancy

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Background and aims: Pregnancy is associated with progression of diabetic retinopathy and therefore frequent retinal screening to allow prompt treatment of disease is necessary. Our aims were to evaluate if our patients were receiving appropriate retinal screening during pregnancy and to assess the proportion who had progression of diabetic retinopathy. Additionally we wished to identify factors influencing screening and progression of retinopathy in our cohort.

Materials and methods: We identified 341 women with pregestational diabetes from the Atlantic DIP database. This cohort comprised 233 (68%) with type 1 diabetes and 108 (32%) with type 2 diabetes. Screening was deemed appropriate if it occurred at least twice during pregnancy in separate trimesters. Statistical analysis was performed using SPSS version 20.0 (IBM). Hypothesis testing was performed on the data of equal variance and normal distribution using an unpaired Student's t test. A Chi-squared analysis was used to compare sample proportions. Binary logistic regression was used to evaluate factors associated with appropriate screening and progression of retinopathy.

Results: Appropriate screening took place in 191 (56%) pregnancies, more commonly in women with type 1 diabetes. Women who received appropriate screening attended pre-pregnancy care (PPC) [56.5% versus 22.7% (p<0.001)] and received folic acid [69.6% versus 53.3% (p=0.002)] more frequently than those who did not. Women who received appropriate screening also had a longer duration of diabetes [11.3 ± 2.9 versus 9.3 ± 2.9 years (p=0.03)]. There was a significant difference in rates of adequate screening across the five antenatal centres participating in the Atlantic DIP study (21.6% - 71.4%, p<0.001). Modelling by logistic regression identified PPC as the only maternal factor significantly associated with re-

ceiving appropriate screening [odds ratio 4.01; CI 2.38–6.75 ($p < 0.001$)]. On evaluation of those patients who received appropriate screening ($n = 191$), it was noted that 49 (26%) had retinopathy progression during pregnancy. Univariate analysis revealed that these women had on average, a longer duration of diabetes [14.4 ± 5.7 versus 10.0 ± 2.8 years ($p = 0.003$)], a higher 1st trimester HbA1c [7.7 ± 1.6 versus $7.1 \pm 1.4\%$ ($p = 0.02$)], a larger drop in HbA1c between the 1st and 3rd trimesters of pregnancy [1.4 ± 1.3 versus $0.7 \pm 0.9\%$ ($P = 0.002$)] and a higher systolic blood pressure at the booking visit [128.0 ± 17.6 versus 122.2 ± 13.2 mmHg ($p = 0.02$)] compared to women with no progression. Logistic regression analysis of significant factors from univariate analysis, revealed decrease in HbA1c [odds ratio 2.09; CI 1.11; 3.92 ($p = 0.02$)] and systolic blood pressure at booking [odds ratio 0.03; CI 1.03; 1.06 ($P = 0.05$)] as significant factors associated with retinopathy progression in pregnancy.

Conclusion: We demonstrate inadequate screening for diabetic retinopathy during pregnancy and significant variation across participating centres. Our study highlights the importance of participation in PPC as this is associated with appropriate screening in the ensuing pregnancy. As 26% of women continue to demonstrate progression of retinopathy during pregnancy, there is urgent need to ensure adherence to screening protocols. This is particularly important for women with larger reductions in HbA1c during pregnancy and higher systolic blood pressure at booking as these women are at greatest risk for progression.

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Association of stillbirth with type 2 diabetes development and future cardiovascular events in women with and without gestational diabetes: a population-based cohort study

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Background and aims: Women with gestational diabetes mellitus (GDM) are at increased risk for the onset of type 2 diabetes (T2DM) and cardiovascular disease in the years following pregnancy. Aim of the study was to estimate the incidence of T2DM and cardiovascular events in women with previous GDM and in those with normal glucose tolerance in pregnancy and to evaluate the role of stillbirth in differentiating the risks.

Materials and methods: A population-based cohort study using administrative data of 12 local health authorities in Puglia, Italy, during the index period from January 1, 2002, to December 31, 2010 was conducted. From a population-based sample of 2.1 million women we identified those with a diagnosis of GDM during the index period and they were propensity-matched on a 1-to-3 basis with women without GDM or diabetes mellitus. Characteristics that were matched were age, local health authority code, use of antihypertensive and antithrombotic agents. Main outcome measures were T2DM development and hospitalizations for cardiovascular events occurring after a pregnancy complicated by GDM and ended at term or in miscarriage.

Results: There were 3851 women with GDM (mean age 37.1 ± 5.9 years) and 11553 matched controls without GDM. During a median follow-up of 5.4 years, the incidence rate of T2DM was of 2.1 per 1000 person-years in women without GDM, of 54.0 per 1000 person-years among women with GDM and pregnancy at term, and 115.0 per 1000 person-years among women with GDM and a pregnancy ended in stillbirth. The cumulative IRs of T2DM development showed that GDM increased the risk of T2DM by 21.7 times, while GDM complicated by stillbirth increased the risk of T2DM by 46.9 times as compared with women with a normal pregnancy. GDM and stillbirth during GDM were associated with a significantly higher risk of cardiovascular events compared with normal pregnancy (IRR, 2.4; 95% CI, 1.5 to 3.8 and IRR, 16.7; 95% CI, 3.7 to 74.7, respectively).

Conclusion: Pregnancy complicated by GDM and ended in stillbirth represents a decisive factor in determining the development of T2DM and future cardiovascular events. For this reason these women deserve a careful follow-up.

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Maternal obesity, glucose levels in early pregnancy, and gestational diabetes in association with cognitive and psychomotor development at 4 years of age

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Background and aims: Fetal life and early infancy are critical periods for brain development. An hyperglycaemic environment in utero may programme biological regulatory mechanisms inducing delay in brain maturity and neurobehavioural abnormalities in offspring. Few studies have examined associations of maternal obesity and diabetes in pregnancy with offspring neurodevelopment, with conflicting results. The aim of this study was to assess the associations of maternal glucose and insulin levels in early pregnancy, gestational diabetes (GDM), and pre-pregnancy body mass index (BMI) with offspring cognitive and psychomotor development at 4 years of age.

Materials and methods: The mother-child “Rhea” study in Crete is a prospective cohort examining pregnant women (Greek and immigrants) residents at the prefecture of Heraklion that became pregnant during one year starting in February 2007. The present analysis includes seven hundred seven (707) mother-child pairs, after excluding twin pregnancies, and women with pre-gestational diabetes. Maternal fasting serum samples were collected at the time of the first major ultrasound (Mean: 12 weeks, SD: 1.5). Pregnant women were screened for GDM between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Neurodevelopment at 4 years was assessed by means of the McCarthy Scales of Children's Abilities (MSCA). Multivariable linear regression models were used to estimate the effect of maternal glucose and insulin levels in early pregnancy, GDM, and pre-pregnancy BMI on neurodevelopmental scores at 4 years of age after adjusting for maternal age, education, parity, child sex, birth weight, breastfeeding duration and quality of assessment.

Results: The percentage of overweight and obese mothers prior to gestation was 21% and 12.7% respectively, while 8% women developed GDM. Maternal obesity prior to gestation was associated with reduced general cognitive (score reduction: -2.33; 95% CI: -4.57, -0.10), and perceptual development scores (score reduction: -2.87; 95% CI: -5.19, -0.55) at 4 years of age. There was also evidence of a dose-response relationship with continuous maternal BMI. Stratified analysis by child sex showed a higher reduction in girls compared to boys (general cognitive score reduction: -4.00; 95% CI: -7.25, -0.75; perceptual score reduction: -3.42; 95% CI: -6.57, -0.27, p for interaction < 0.01). Gestational diabetes, and maternal fasting glucose and insulin levels in early pregnancy were not associated with offspring neurodevelopmental outcomes after adjustment for potential confounders.

Conclusion: Maternal obesity prior to gestation may contribute to reduced child cognitive development at preschool age, while GDM was not found to be significantly associated with offspring neurodevelopment. Further follow up of this cohort will allow to determine whether these findings persist into later childhood development.

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Gestational diabetes mellitus and risk for autism in offspring

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Background and aims: Pregnancies complicated by gestational diabetes mellitus (GDM) increase risks of perinatal morbidities and postnatal obesity and metabolic disorders in offspring. A few studies also suggest that maternal hyperglycaemia during pregnancy is associated with a high rate of inattention, developmental delays, and autism spectrum disorders (ASD) in offspring. The hypothesis is that hyperglycaemia may “disrupt” fetal brain development and maturation and lead to increased risks for offspring to develop neurobehavioral disorders in later life. We conducted a large population-based study

to assess the association between GDM exposure *in-utero* and development of ASD in offspring.

Materials and methods: This retrospective longitudinal cohort included children who were born as a singleton at 28–44 weeks gestation in hospitals from a large integrated health plan between January 1, 1995 and December 31, 2009. Children were required to have the health plan membership by age 1–2 years, when screening for developmental delays and ASD are initiated per the health plan guidelines. Children were prospectively followed from birth using electronic medical record until any one of following criteria was met: (1) a clinical diagnosis of ASD identified by ICD-9 codes 299.x, (2) >4-months of inactive health plan membership, (3) death from any cause, or (4) December 31, 2012. Children who were born to women with pre-existing diabetes were excluded. Survival analysis was used to estimate the incidence rate of ASD and Cox proportional hazard regression was used to estimate the relative risk (RR) of developing ASD associated with GDM.

Results: A total of 336,164 children (26,897 [8%] GDM exposed) met the cohort inclusion criteria with a median 5.5 years (range 1.0–18.0 years) of follow-up during which 4,526 children developed ASD. The incidence rate of ASD was 2.36/1000 per year (2.81/1000 for GDM and 2.32/1000 for non-GDM group, respectively). In the unadjusted analysis, the incidence of ASD in the GDM exposed group was 20% higher than that in the GDM unexposed group (RR= 1.20, 95% CI: 1.08–1.32, $p=0.0004$). However, the RR was reduced to 1.06 (95% CI: 0.96–1.18, $p=0.22$) after adjustment for maternal age, parity, education, household income, race/ethnicity and child gender. The greatest reduction in the RR was due to the adjustment for maternal age (from 1.20 to 1.09) as mothers who had GDM were on average 3.2 years older at delivery than mothers without GDM. Additional adjustment for child birth weight, gestational weeks at delivery and congenital abnormalities at birth, factors known to be associated with maternal hyperglycaemia, slightly reduced the RR association between GDM and ASD (to 1.04, $p=0.46$). Excluding children with congenital abnormalities at birth had no impact to the results. Analysis by ASD sub-type reached similar conclusions.

Conclusion: Data from a large multi-ethnic and population-based clinical care system did not support significant association between GDM exposure *in-utero* and risk of development of ASD in offspring. The slightly elevated risk for ASD in offspring exposed to GDM was largely due to older age of women with GDM.

OP 27 Protecting the periphery

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mRNA expression profiling in perivascular and subcutaneous adipose tissue of patients with carotid artery (ACI) stenosis

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Background and aims: There is growing evidence for a vasoregulatory and atherosclerosis-inducing role of local fat deposits around vessels. Secreted adipokines may contribute directly or indirectly to the regulation of vessel wall homeostasis. Investigating molecular mechanisms specific for perivascular fat could therefore contribute to better understanding of its role in the pathophysiology of atherosclerosis and its sequelae such as stroke or myocardial infarction. Aim of the study was to investigate gene expression profiles in paired human samples of subcutaneous (sc) and perivascular (pv) adipose tissue (AT) and to link it to clinical and anthropometric characteristics of carotid stenosis patients.

Materials and methods: A RNA/cDNA bank was established from paired sc (cervical) and pv (ACI) AT samples of patients who underwent carotid endarterectomy. Tissue specific marker genes (e.g. *MYH2* for muscle, *COMP* for fibroblasts or *GPM6A* for nerve) have been measured to control for impurities of “non-adipocyte” cells. The expression median with appropriate ranges for every marker gene was set specifically for each fat depot. Sixty paired samples passed these criteria and were assayed on Illumina HT12 microarrays. *P*-values for differential expression between AT depots and phenotypic groups (e.g. symptomatic vs. asymptomatic) were calculated using background-corrected, quantile-normalized expression values and paired/standard *t*-test. To correct for multiple testing an experiment based genome wide significance level and FDR methodology were applied. Gene expression was correlated to anthropometric, metabolic and clinical parameters. Best hits underwent validation using TaqMan qPCR technology.

Results: We found 1100 genes with significant differential expression between sc and pv AT clearly distinguishing both AT types from each other. The top hits with >2 fold changes are represented by developmental genes like *HOX* genes, *TBX15* or *WNT5A* but also genes found to be involved in atherosclerosis (*CDKN2B*, *TRIB1*) and coronary artery disease (*PTGS2*, *CARD8*). Intra-depot comparison of e.g. symptomatic vs. asymptomatic, lean vs. obese or diabetic vs. non-diabetic patients revealed genes with nominal differences in mRNA levels, which however, correlated with anthropometric and metabolic parameters (BMI, triglycerides, cholesterol, percent stenosis).

Conclusion: Our data revealed fat depot-specific mRNA expression of developmental genes and genes associated with atherosclerosis or coronary artery disease, supporting the relevance of perivascular adipose tissue in the pathogenesis of atherosclerosis.

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rs196462, near SMOC2, is associated with lower extremity arterial disease in patients with diabetes

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Background and aims: Lower extremity arterial disease (LEAD) is a common macrovascular complication of diabetes and the most common cause of amputations in patients with diabetes. LEAD in patients with diabetes is

often accompanied by dyslipidaemia, renal disease and neuropathy. Despite large sample sizes, previous GWA studies have identified 1 genome wide significant ($p < 5 \times 10^{-8}$) signal near *CHRNA3* and another highly significant signal ($p < 5 \times 10^{-7}$) in the 9p21 region. The lack of strong associations may be due to the highly polygenic architecture of LEAD, phenotypic heterogeneity or both. Based on the different risk factors contributing to LEAD in patients with and without diabetes we performed stratified analyses of LEAD in patients with and without diabetes and in smokers vs. non-smokers, irrespective of diabetes status.

Materials and methods: We combined summary statistics in a fixed effects meta-analysis for 2,356,286 SNPs from 4,544 LEAD cases and 30,404 LEAD controls - of which 2,345 cases and 8,706 controls were patients with diabetes and 3,535 cases and 20,212 controls were smokers. We also performed an interaction analysis of allelic effects in patients with diabetes vs. individuals without diabetes and in smokers vs. non-smokers.

Results: Rs196462, near *SMOC2*, was associated with LEAD in patients with diabetes at genome wide significance ($p < 5 \times 10^{-8}$). Rs10743273, near *PIK3C2G*, was associated with LEAD in non-diabetic individuals and showed evidence for interaction ($OR = 1.2$, $p = 6.3 \times 10^{-4}$, $phet = 6.1 \times 10^{-4}$). Rs2451819 ($OR = 1.2$, $p = 1.2 \times 10^{-5}$, $phet = 5.9 \times 10^{-4}$) near *PIK3R1* and rs7084667 ($OR = 1.3$, $p = 3.2 \times 10^{-5}$, $phet = 6.5 \times 10^{-4}$), near *SFTPD* were associated with LEAD in patients with diabetes and showed evidence for interaction. These genes play a role in insulin resistance, obesity and cardiovascular disease. The top SNP in smokers was rs1051730 ($p = 7.2 \times 10^{-7}$), near *CHRNA3*, that is established for LEAD and is associated with pack years. Rs2076156 ($OR = 2.4$, $p = 1.1 \times 10^{-4}$, $phet = 3.5 \times 10^{-6}$), near *BIK*, rs12593396 ($OR = 1.46$, $p = 4.2 \times 10^{-6}$, $phet = 1.3 \times 10^{-5}$), near *ADAMTS7* and rs11214800 ($OR = 1.3$, $p = 2.6 \times 10^{-4}$, $phet = 2.7 \times 10^{-5}$), near *HRT3A* were associated in LEAD in smokers and showed evidence for interaction with smoking status. The expression of *BIK* is directly affected by cigarette smoke, while SNPs near *ADAMTS7* have been shown to interact with smoking status to affect blood pressure and SNPs in *HRT3A* have been associated with smoking quantity.

Conclusion: Diabetes and smoking are well established risk factors for LEAD and by stratifying analyses by these risk factors we have identified a signal near *SMOC2* that appears to be specific to LEAD patients with diabetes. The interaction analysis also revealed interactions near genes that affect cardio-metabolic traits. While signals in the smoking interaction analysis did not achieve

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The association between diabetic peripheral neuropathy and peripheral artery disease

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Background and aims: Diabetic peripheral neuropathy (DPN) and peripheral artery disease (PAD) are common complications that occur more frequently in patients with type 2 diabetes than in the general population. Data on the association between these two conditions is surprisingly scant. We studied the association between DPN and PAD, including characteristics that were associated with each condition.

Materials and methods: We searched the electronic medical records of 37,095 members of Kaiser Permanente Northwest who had type 2 diabetes identified prior to 2013 and had complete health plan eligibility in 2012 for physician-coded ICD-9-CM diagnoses of DPN or PAD in 2010-2012. We compared demographic (age, sex, diabetes duration) and clinical characteristics (BMI, HbA_{1c} , blood pressure, lipids, and presence of ischemic heart disease [IHD], chronic kidney disease [CKD] and heart failure [HF]) associated with the presence of one or both of these conditions. To isolate the independent association between DPN and PAD, we constructed a multiple logistic regression model that controlled for risk factors for DPN and PAD.

Results: Of the 37,095 type 2 diabetes patients, 24% ($n = 9,044$) had DPN without PAD, 3% ($n = 994$) had PAD without DPN, 4% ($n = 1,592$) had both DPN and PAD, and 69% ($n = 25,465$) had neither condition. The table displays characteristics of patients with neither, both, or either DPN or PAD. Among the 29% of patients with DPN ($n = 10,636$), 15% had PAD compared with 4% without DPN ($p < 0.001$). Nearly two-thirds of patients with PAD also had DPN, while only about one-quarter of patients without PAD had DPN (62% vs. 26%, $p < 0.001$). Characteristics of patients without PAD differed markedly depending on whether DPN was present. For example, mean age of patients with DPN but no PAD was 67.3 years, compare with 59.9 years among those without DPN or PAD ($p < 0.001$), and those with DPN were two to three times

more likely to have IHD, CKD or HF. However, characteristics including age, sex and presence of comorbidities were similar among patients with PAD regardless of the presence of DPN. After controlling for demographic and clinical characteristics, presence of DPN more than doubled the probability of having PAD ($OR = 2.32$, 95% CI 2.08-2.60).

Conclusion: Our results demonstrate a strong association between DPN and PAD that remained even after controlling for a number of characteristics. Although our design did not evaluate the temporal relationship between these two conditions, older age and longer duration of diabetes among PAD patients suggests that DPN may often precede PAD. Research is needed to determine whether treating DPN can reduce the risk of PAD.

	Neither DPN nor PAD	DPN Only	PAD Only	Both DPN and PAD
n	25,465	9,044	994	1,592
%	68.6%	24.4%	2.7%	4.3%
Mean Age, years	59.9	67.3	73.3	73.4
% Men	49.2%	51.7%	56.5%	58.6%
Duration of diabetes, years	6.2	9.9	8.3	12.8
Hemoglobin A1c (%)	7.3%	7.4%	7.0%	7.2%
Hemoglobin A1c (mmol/mol)	56	57	53	55
Systolic Blood Pressure	131	132	133	132
Ischemic Heart Disease	12.7%	24.0%	49.3%	51.4%
Chronic Kidney Disease	14.0%	29.4%	38.8%	47.9%
Heart Failure	5.2%	14.9%	25.5%	34.9%

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Clinical and ultrasonographic results of endovascular therapy in diabetic patients with critical limb ischaemia

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Background and aims: To evaluate arterial patency and clinical outcomes after percutaneous transluminal angioplasty (PTA) in diabetic patients with critical limb ischemia (CLI).

Materials and methods: From September 2010 to June 2013, a prospective single-center study was conducted involving 164 diabetic patients with CLI (76(46%)) men, mean age 64.1[54-68] years, mean HbA_{1c} 7.9±1.4%, mean duration of diabetes 16.5[0.8-43] years, diabetes type 1/2 - 16/148 who underwent PTA in 193 limbs. Myocardial infarction and stroke in anamnesis were in 25(15%) and 15(9%) patients, respectively. Chronic kidney disease (CKD) 3-4 stages was in 40(24%) patients, end-stage renal disease 16(10%). Diagnosis of CLI based on recommendation of TASC II. Patency of arteries of lower extremity was evaluated by duplex ultrasound (DU) during whole follow-up period. PTA in all patients was considered technically successful in restoring continuous arterial flow to the foot of at least one crural artery without residual stenosis > 50%. Follow-up assessment during 3 years included clinical examination for wound healing (WH), limb salvage (LS) and common survival (CS).

Results: Peripheral arterial disease 4 category according Rutherford classification revealed in 29(15%), 5 category-in 103(53.4%), and 6 category in 61(31.6%) patients. 0-1 the degree of tissue damage according Wagner classification identified in 29 (15%) cases, W 2 - 100 (51.8%) cases, W 3-4 - 64 (33.2%) cases. Peripheral arterial disease 4-6 classes according Graziani classification was in 180 (93%) cases. Extensive tibial arterial calcification was in 123(64%) cases, in patients with residual stenosis (> 50% remaining diameter)-113 (89%). After PTA residual stenoses (>50%) in treated arteries were in 125(79.1%), thrombosis in the treated arteries - 9(5.7%), intimal dissection - 18(11.4%), incomplete stent disclosure - 3 (1.9%), incomplete capture stent area stenosis - 2(1.3%) and dislocation of the stent - 1(0.6%). Repeat PTA in the early period (30 days) was in 15 patients. Cumulative primary patency in the femoropopliteal segment was 55%, in tibial segment 25%; in patients with residual stenosis in treated arteries <50% - 21%, with residual stenosis ≥50% - 66% (Kaplan-Meier Estimates). Repeat PTA during follow-up was performed in 73(38%) cases generally, in patients with CKD 3-5 stages 18(45%). There were 10(5.2%) major amputations (log-rank, $p < 0.05$), in patients with end-stage renal disease 5(2.6%); LS rate was 94%, WH rate was 98% (log-rank, $p < 0.05$); CS was 81% (log-rank, $p < 0.05$).

Conclusion: CLI in diabetic patients is characterized by having severe morphological lesions of the lower limb arteries, arterial calcification and soft tissue lesions. The high level of residual stenosis was revealed after PTA. Extremely low primary patency in diabetic patients with CLI mainly at the tibial

arteries is associated with chronic renal insufficiency and residual stenosis. Timely reintervention in diabetic patients with recurrent CLI promotes optimal WH, LS and CS.

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Optimal blood pressure targets for prevention of peripheral artery disease in patients with type 2 diabetes and hypertension

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Background and aims: Peripheral artery disease (PAD) is one of the macrovascular complications of diabetes, and often associated with considerable functional limitation. Although the benefits of reducing high blood pressure (BP) on the risks of cardiovascular disease and cerebral artery disease are well known, the optimal BP goal is not established. The guideline recommended BP target for patients with diabetes had recently been changed from <130/80 mmHg to <140/80 mmHg, because there have been no clear evidence showing that tight control (<130/80 mmHg) was associated with improved cardiovascular outcomes compared with <140/80 mmHg. However, previous studies did not address which goal is more appropriate for the reduction of PAD events. In this study, we retrospectively examined whether subjects with the tighter BP control were associated with better clinical outcomes with respect to PAD events in patients with type 2 diabetes mellitus and hypertension.

Materials and methods: Among 963 patients with type 2 diabetes and hypertension, referred to our department between January 2000 and December 2012 (633 for men, mean age 66 years, HbA1c 8.7%, mean BP 137/74 mmHg), subjects with baseline BP<140/80 mmHg were selected (n=513). Patients were categorized into two groups by their BP at baseline: Group 1, less than 130/80 mmHg and Group 2, 130/80 mmHg or higher. As a result, 262 patients (51%) were categorized into Group 1 (33% for men, mean age 66 years), and 251 patients (48%) into Group 2 (32% for men, mean age 66 years). The effect of baseline BP category on the development of PAD event was analyzed at a mean follow-up of 4.5 ± 3.1 years. Multivariable Cox regression models were performed to examine the independent association between baseline BP category and PAD events during follow-up. The PAD event was defined as angioplasty for PAD or ischemic ulcer requiring hospital care. **Results:** Among all subjects, mean systolic and diastolic BP of Group 1 were 116 ± 7.5 mmHg and 64.8 ± 7.2 mmHg respectively, whereas those of Group 2 were 131.0 ± 14.2 mmHg and 74.5 ± 10.5 mmHg respectively. Between the two groups, there were no significant difference with respect to age, sex, smoking, HbA1c, eGFR, insulin use, previous stroke and previous myocardial infarction. However the prevalence of PAD at baseline was significantly higher in Group 2 (8.8 % in Group 1 vs. 14.7 % in Group 2, $p=0.04$). During follow-up, 8 patients (1.6% of total population) were hospitalized for PAD events. Kaplan-Meier estimation showed that the risk of PAD hospitalization was significantly higher in Group 2 (log-rank $p=0.03$). Cox regression analysis revealed that the association between baseline BP category and PAD events was significant after adjustment of age, sex, smoking, HbA1c, eGFR, insulin use, and previous stroke, myocardial infarction and PAD at baseline ($p=0.04$).

Conclusion: In patients with type 2 diabetes and hypertension, baseline BP<130/80 mmHg showed significantly lower risk of future PAD events than BP<140/80 mmHg. It is, therefore, suggested that tighter control of blood pressure could reduce the incidence of PAD events in patients with type2 diabetes and hypertension.

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Beneficial effect of multifactorial intervention on the prognosis of patients with type 2 diabetes mellitus and critical limb ischaemia/peripheral arterial disease

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Background and aims: The combination of critical limb ischemia/peripheral artery disease (PAD) and diabetes mellitus type 2 (T2DM) is known for poor survival. The last major publication on successful management of such a patient population reported a 50% mortality and 25% amputation rate after six years of follow up. We have analyzed whether more recent treatment advances of T2DM and PAD in the last five years have ameliorated those detrimental effects.

Materials and methods: In a prospective study we enrolled 366 patients (34% female) with PAD, 38% had T2DM, 33% impaired glucose tolerance (PRE) and 29% normal glucose tolerance (NGT). As expected the patient cohort had a high cardiovascular risk factor (CRF) burden: 92% hypertension, 97% hyperlipidemia, 74% active or former smoker; Coronary heart disease (CHD) was known in 32% and carotid artery disease (CAD) in 39% of the patients. Within 6 months the target values of CRF control - LDL-Cholesterol <100 mg/dl, blood pressure (<140/80 mm Hg, HbA1c in DM <7.0%) were reached in 58%, 69% and 69%. Patients followed a strict control visit program in the center for 5 years.

Results: The overall survival of this cohort was 89.3% after 4.9 years. MACE (combination of death, non-fatal myocardial infarction or stroke) free survival was 84.3% and event free survival including interventional or surgical procedures due to critical limb ischemia/PAD was 68%. Patients with T2DM showed a survival of 87.8% compared to 89.3% PRE, and 95.2% NGT ($p=0.161$). MACE free survival was 81.3% for T2DM, 87.6% for PRE, and for 92.4% NGT ($p=0.059$). Additionally, event free survival was 65.5% for T2D, 71.9% for PRE, and 77.1% for NGT ($p=0.155$).

Conclusion: In summary, strict multifactorial management induced a dramatic reduction in the annual death rate (2.8 for patients with T2DM and PAD), MACE and amputation. Thus, management of such patients should be restricted to centralized centers to improve outcome for the patients.

OP 28 Ectopic lipids and type 2 diabetes

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Effect of bariatric surgery on hepatic fatty acid uptake and blood flow

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Background and aims: Increased delivery of free fatty acids (FFAs) to the liver plays a role in the pathogenesis of type 2 diabetes. Bariatric surgery is associated with high rates of diabetes remission, but the underlying mechanisms are not fully elucidated. Liver fatty acid uptake is crucial in understanding the mechanism of postoperative metabolic changes. This study's objective was to assess the surgery-induced changes in hepatic FFA uptake, together with quantification of hepatic perfusion and liver fat content (LFC).

Materials and methods: We measured hepatic FFA uptake, hepatic portal, and arterial perfusion with positron emission tomography using [18F]-FTHA and [15-O]-H₂O in 22 morbidly obese subjects before and 6 months after bariatric surgery and in 14 healthy lean volunteers. LFC was quantified by MRS and liver volume by MRI.

Results: Baseline: Plasma FFA concentration was elevated in the obese group compared to the lean control group (0.67 ± 0.22 vs. 0.48 ± 0.21 mmol/l, $p = 0.02$). Liver FFA uptake, when measured per depot or per unit mass, was higher in obese subjects compared to controls (depot: 254 ± 67 vs. 107 ± 39 μ mol/min, $p < 0.001$ and per unit mass: 14.4 ± 3.6 vs. 10.0 ± 3.7 μ mol/min/100 ml, $p < 0.01$, respectively). LFC in the obese group was significantly higher than in controls (5.7 ± 4.7 vs. $1.5 \pm 1.5\%$, $p < 0.01$), and 9 of the obese patients had liver steatosis (LFC $\geq 5\%$). When obese subjects were divided into two groups, with LFC below (LFC-low) or above (LFC-high) the median of 4.4%, the LFC-high group had an increased FFA depot uptake compared to LFC-low group ($p = 0.09$). When patients were grouped according to diabetic status, no difference in FFA uptake was observed. In pooled data, FFA uptake correlated with intraperitoneal fat mass ($r = 0.40$, $p = 0.02$). Compared to controls, both portal and arterial blood flow were higher in the obese group (5.2 ± 2.1 vs. 3.6 ± 1.0 l/min, $p = 0.01$ and 0.34 ± 0.16 vs. 0.19 ± 0.10 l/min, $p < 0.001$, respectively). At 6 months after surgery: BMI decreased from 41 ± 4 kg/m² to 32 ± 4 kg/m² ($p < 0.001$) and the remission of diabetes was observed in 8 out of 10 patients. Plasma FFA concentration was unchanged ($p = \text{NS}$ vs. baseline). The liver depot uptake decreased by 15% ($p = 0.02$ vs. baseline), but remained twice as high compared to the control group ($p < 0.001$ vs. controls). LFC was decreased by 69% ($p = 0.001$ vs. baseline) and was now similar to that of lean controls ($p = \text{NS}$ vs. controls). None of the subjects had liver steatosis postoperatively. Portal blood flow decreased by 55% ($p < 0.0001$) and arterial flow by 76% ($p < 0.0001$), and both were reduced compared to levels measured in controls ($p < 0.05$ for both). Reduction in intraperitoneal fat mass was related to changes in LFC ($r = 0.59$, $p = 0.02$) and in portal blood flow ($r = 0.69$, $p = 0.001$). An association between the change in portal blood flow and improvement in insulin sensitivity index was observed ($r = 0.52$, $p = 0.02$).

Conclusion: Hepatic fatty acid uptake was decreased, but did not normalize to controls, when measured six months after bariatric surgery. After surgery, fatty acid uptake changes may contribute to the resolution of liver steatosis. Morbidly obese subjects showed increased portal vein blood flow, which resulted in a higher supply of fatty acids for hepatic FFA uptake. Postoperatively blood flow decreased along with the reduction in visceral fat mass and improvement in insulin sensitivity.

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High-fat diet increases autophagic flux in pancreatic beta cells in vivo

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Background and aims: Autophagy is an important cellular survival mechanism that responds to changes in cellular nutrients, and potentially counter-regulates endoplasmic reticulum (ER) stress, previously implicated in

lipotoxic beta-cell death. Because it is extremely difficult to measure true autophagic flux in vivo, it is still unclear how high-fat feeding or fatty acids regulate autophagy in pancreatic beta-cells. Here, we used both in vivo and in vitro models to study autophagic flux; and also investigated the upstream signaling pathways triggered by fatty acids.

Materials and methods: For unequivocal estimation of autophagic flux, GFP-LC3 mice were fed with chow or high-fat diet for 10 weeks and then, for 5 consecutive days before sacrifice, injected with 100mg/kg chloroquine to block clearance of autophagic markers. The pancreata and livers were collected and fixed for cryosectioning. The beta-cells of the pancreatic sections were labeled with the insulin antibody, and the GFP-LC3 signaling analysed by fluorescence microscopy. Autophagic and ER stress markers were detected by western blot following acute (2h) treatment with chloroquine using both islets ex vivo from the high-fat fed mice, and mouse clonal MIN6 beta-cells treated with oleate and palmitate for 0–48h.

Results: Autophagic flux, assessed from the amount of GFP puncta, was increased in pancreatic acinar tissue and beta-cells after high-fat feeding and chloroquine injection. In contrast, GFP signal in the liver was markedly attenuated under identical conditions. LC3 levels in the isolated mouse islets were also increased by high-fat diet and further enhanced by chloroquine incubation ex vivo. ER stress, indicated by the markers CHOP and phospho-eIF2 α levels, in isolated islets was not augmented by high-fat feeding alone, but was induced by autophagic inhibition with chloroquine and further enhanced by high-fat feeding. This reveals a reciprocal relationship between ER stress and autophagy in beta-cells and suggests that autophagy is not solely secondary to ER stress in the context of lipotoxicity in vivo. In MIN6 cells, oleate increased LC3-II levels in both the presence and absence of chloroquine (indicative of enhanced flux and steady state autophagy respectively) whereas palmitate augmented the autophagic flux alone. ER stress was increased by palmitate but not oleate, which concurs with the in vivo model that lipids/fatty acids could induce autophagy without the activation of ER stress. Nevertheless, neither fatty acid modulated classical mTOR signaling illustrated by the downstream phospho-4EBP levels.

Conclusion: Using GFP-LC3 mice with chloroquine injection we show for the first time that beta-cells have a very low basal autophagic flux but, in contrast to liver, this is increased by high-fat feeding. This appears to act as a protective mechanism prior to the activation of ER stress, and is triggered independently of the classical mTOR pathway.

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Sphingosine kinase 1 promotes hepatic steatosis via up-regulation of PPARGamma

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Background and aims: Sphingolipid metabolites have emerged playing key roles in the pathogenesis of metabolic diseases, including obesity, diabetes and nonalcoholic fatty liver disease (NAFLD). The current study aimed to explore the underlying mechanisms and discover a potentially druggable intermediate in the steatotic pathways associated with sphingolipid metabolites.

Materials and methods: We utilized pharmaceutical inhibitors or genetic means to manipulate sphingosine kinase 1 (SphK1), one of the key enzymes that control sphingolipids metabolism. Both cellular and animal models were applied in the study investigating the role of SphK1 in hepatic lipid metabolism and NAFLD development.

Results: A nearly 2-fold increase in the expression level of SphK1 was observed in either cellular or animal models of NAFLD, without alterations in SphK2 (another isoenzyme of SphK), suggesting a specific link of SphK1 to NAFLD. Enforced overexpression of SphK1 significantly promoted lipid accumulation in mouse primary hepatocytes, whereas loss of SphK1 gene expression inhibited the process compared with the control hepatocytes (both $p < 0.01$). Moreover, SphK1 deletion resulted in a significant amelioration in histological changes of steatosis, along with a 30% reduction in hepatic contents of triglycerides and cholesterol in diet-induced obese (DIO) mice ($p < 0.01$). Analysis of fatty liver-related gene expression revealed a 40% reduction of transcriptional factor peroxisome proliferator-activated receptor γ (PPAR γ) and its target genes in DIO SphK1^{-/-} mice, in comparison with wild-type DIO mice ($p < 0.01$). Accordingly, treatment with sphingosine-1-phosphate (S1P) or overexpression of SphK1 increased PPAR γ expression by 150% ($p < 0.01$) in the lipid-laden hepatocytes. Furthermore, PPAR γ inhi-

bition by using its siRNA or antagonists markedly reduced SphK1-dependent hepatic lipid accumulation (all $p < 0.01$).

Conclusion: SphK1 plays an important role in fatty liver development by promoting lipid accumulation and up-regulation of PPAR γ in hepatocytes. The functional link between the SphK1 and PPAR γ pathways in aggravating hepatic steatosis may reveal a new way to the management of NAFLD.

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Hypothalamic nitric oxide regulates insulin signalling and hepatic lipid metabolism

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Background and aims: Human studies demonstrated that NO is an important regulator of insulin clearance. Whether the control of insulin clearance is solely dependent on hepatic mechanisms or also involves hypothalamic regulation is currently uncertain. Since hypothalamus is a critical regulator of energy metabolism and endocrine functions, we previously hypothesized that NO production by central/hypothalamic axis regulates insulin clearance and therefore peripheral insulin bioavailability. We found a significant inverse correlation between NO production in the paraventricular nucleus (PVN) and systemic insulin clearance. However, the metabolic consequences of PVN NO-mediated regulation of insulin clearance remain unknown, so we hypothesized that in addition to insulin clearance, it also modulates hepatic glucose and lipid metabolism.

Materials and methods: Male Wistar rats underwent brain surgery using a stereotaxic apparatus for implantation of the double cannulas, for nucleus specific infusion in the PVN region. After the bregma localization the following coordinates were used: AP: -1.8mm, Lat: +/- 0.4mm, DV: -8.0mm. Bolus infusion of 250ug/2uL of L-NAME (or 2uL of saline in the control animals) were performed in each side of the brain, once only in the day of the experiment. An oral glucose tolerance test (OGTT) (2g/kg) was subsequently performed. Liver was harvested and kept at -80° for protein extraction and analysis of glucose and lipid metabolism-related enzymes by immunoblotting.

Results: We observed that PVN NO depletion led to changes in insulin signaling as well as glucose and lipid metabolism. Regarding insulin signaling, IRS-1 and AKT expression as well as IRS-1 and AKT phosphorylation were decreased. In addition, expression of hepatic Glut 1, 2 and 4 were all significantly decreased suggesting a reduced capacity for glucose uptake by the liver. Interestingly, depletion of PVN NO levels suppressed the lipogenic enzymes (ACC, FAS, ATP citrate lyase and pATP citrate lyase), which is consistent with the observed diminished hepatic non-esterified fatty acids (NEFA) content. Meanwhile, hepatic DGAT expression was increased, possibly in response to decreased NEFA. Hepatic TG levels were not diminished by PVN-NO depletion possibly due to this compensatory DGAT upregulation.

Conclusion: These results support the hypothesis that increased PVN NO results in a decreased insulin clearance and a stimulation of de novo lipogenesis. This suggests that hypothalamic NO signaling has a role in regulating hepatic lipid metabolism.

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MiR-494 is regulated by exercise in obese sedentary individuals with increased risk of type 2 diabetes and modulates skeletal muscle lipid metabolism in vitro

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Background and aims: Exercise leads to activation of several signaling pathways which improve skeletal muscle lipid metabolism and insulin sensitivity. On the contrary, physical inactivity is associated with impaired lipid metabolism and insulin resistance. We hypothesized that miR-494 might be involved in the response of skeletal muscle to both acute and regular exercise. The aim of our study was to investigate regulation of miR-494 in skeletal muscle of sedentary overweight to obese individuals (age 36.0 ± 1.7 yrs; BMI 31.6 ± 0.9 kg.m⁻²; M/F, 8/7) with increased risk of T2D (prediabetics, n=10) who underwent a 12-week endurance (n=8) or strength (n=7) training programme. A functional study examining the role of miR-494 in regulation of lipid metabolism was performed in primary human myotubes.

Materials and methods: Biopsies of *m. vastus lateralis* were taken prior and after training. Effects of a single exercise bout were examined in a subpopulation of 8 individuals (M/F, 4/4). All participants underwent complex metabolic phenotyping, including an assessment of glucose tolerance (oGTT), subcutaneous and visceral adipose tissue content and distribution (MRI) and maximal aerobic capacity (bicycle ergometry). Total RNA, including miRNA, was isolated from skeletal muscle & serum and quantified with the aid of real-time PCR. *In vitro* functional studies were performed in primary human myotubes, transfected with miR-494 mimics, inhibitor or negative control.

Results: The 12-week training led to 19% increase of VO₂ max ($p < 0.05$), confirming the efficiency of training intervention. Expression of miR-494 in skeletal muscle was reduced by 28% ($p < 0.05$) in response to 12-week training. However, no effect of an acute exercise bout on muscle miR-494 was observed in neither sedentary nor trained individuals. Interestingly, circulating levels of miR-494 were 2.6-fold higher ($p < 0.01$) after 12-week training. Overexpression of miR-494, lowered expression of FoxJ3 (-48%, $p < 0.001$), PGC1 α (-33%, $p < 0.001$), SIRT3 (-18%, $p < 0.001$) and SIRT1 (-36%, $p < 0.001$) and was associated with 22% reduction of mitochondrial content ($p < 0.05$) and marked increase (3.6-fold, $p < 0.001$) in triglyceride accumulation. Transfection of miR-494 inhibitor significantly increased expression of PGC1 α (+26%, $p < 0.001$) and SIRT1 mRNA (+17%, $p < 0.01$) and reduced lipid accumulation (-30%, $p < 0.01$) in differentiated human myotubes. However, no effect of miR-494 inhibitor on mitochondrial content was observed.

Conclusion: Our results indicate that both skeletal muscle and circulating miR-494 levels are regulated by exercise training. In addition, our *in vitro* functional studies suggest that miR-494 might be involved in regulation of mitochondrial biogenesis and lipid storage/utilization capacity, likely by targeting the PGC1 α and SIRT1 pathways.

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Fatty acid composition determines the acute effects of oral fat intake on insulin sensitivity and muscle mitochondrial function in humans

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Background and aims: Intravenous administration of fat, mainly consisting of unsaturated lipids, promotes insulin resistance, which might relate to altered mitochondrial function. However, it remains unclear whether oral fat ingestion may also induce muscle and/or hepatic insulin resistance, and whether fatty acid composition might differently affect insulin sensitivity and skeletal muscle oxidative capacity. Thus, we examined in the present study the acute effects of an oral lipid challenge rich in either saturated or unsaturated lipids on insulin sensitivity and muscle mitochondrial respiration in healthy humans.

Materials and methods: Ten healthy lean sedentary volunteers (age 25 ± 1 years, body mass index 24.8 ± 0.3 kg/m²) were randomized in a cross-over manner to an oral lipid emulsion (50% fat) enriched in saturated fatty acids (SAFA: 92 g palm oil), omega-6 polyunsaturated fatty acids (PUFA: 92 g safflower oil) and water as control (CON). Skeletal muscle biopsies were performed before and 2.5 hours after interventions. Euglycemic-hyperinsulinemic clamps with infusion of deuterated glucose were initiated 6 hours after interventions to assess whole-body (M-value) and hepatic insulin sensitivity (insulin-mediated suppression of endogenous glucose production). High-resolution respirometry was applied to assess ex-vivo muscle mitochondrial respiratory capacity.

Results: After both SAFA and PUFA, whole-body insulin sensitivity was lower compared to CON (CON: 7.3 ± 0.8 ; PUFA: 5.8 ± 0.8 ; SAFA: 4.3 ± 0.5 mg.kg⁻¹.min⁻¹, $p < 0.05$), while hepatic insulin sensitivity remained unaltered (CON: 76 ± 5 ; PUFA: 79 ± 3 ; SAFA: $65 \pm 5\%$). SAFA led to greater whole-body insulin resistance than PUFA ($p < 0.05$), and tended to reduce hepatic insulin sensitivity to a greater extent ($p = 0.06$). Plasma incretin levels rose after both interventions without affecting however serum glucose and insulin levels. Although plasma free fatty acids (FFA) did not change upon SAFA or PUFA, the triglyceride enrichment of circulating plasma chylomicrons was markedly higher after both interventions (total AUC_{0-8h}; CON: 84 ± 13 ; PUFA: 236 ± 52 ; SAFA: 280 ± 65 % hour, $p < 0.05$). SAFA-induced insulin resistance associated with impaired suppression of lipolysis under insulin-stimulated conditions, whereas PUFA-induced insulin resistance related to increased post-intervention plasma FFA levels ($p < 0.05$). ADP-stimulated respiration related to β -oxidation (malate, octanoylcarnitine, ADP) was lowered in muscle after PUFA intake (before: 22.4 ± 2.2 ; after: 18.8 ± 2.5 pmol.sec⁻¹.mg⁻¹, $p < 0.05$), but was not altered after SAFA intake.

Conclusion: Oral intake of both saturated and unsaturated fat induces acutely peripheral insulin resistance in healthy humans, which may be partly mediated by increased circulating triglyceride-rich chylomicrons contributing to muscle lipotoxicity. The underlying mechanisms are divergent depending on fatty acid composition, and may implicate adipose tissue insulin resistance in the case of saturated, and impaired skeletal muscle oxidative capacity due to FFA lipotoxicity in the case of unsaturated fat.

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OP 29 Autoimmune diabetes

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Mass sequencing of the faecal virome: a study in children with early onset of islet autoimmunity

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Background and aims: The search for an association between viruses and islet autoimmunity has been limited mostly to candidate virus genera and serotypes, whereas information has been very scarce about the whole virome (i.e. the complete “virus flora”). The next-generation sequencing technologies have offered a possibility to identify all prevalent viruses in a given sample. We used this technology to contrast the stool virome between children who developed very early islet autoimmunity with subsequent type 1 diabetes, and their matched peers in the Finnish prospective Diabetes Prediction and Prevention (DIPP) birth cohort.

Materials and methods: We conducted a case-control study nested within the DIPP cohort; nineteen cases were selected among children with the earliest onset of islet autoimmunity (mean age 15 months at first islet autoantibodies). Control children without islet autoimmunity were matched 1:1 for gender, time and place of birth and for the HLA risk. The virome was sequenced in available stool samples preceding the onset of autoimmunity by 3, 6 and 9 months. After mechanical enrichment of the virus fraction and nucleic acid extraction, we performed reverse transcription with tagged random primers, second strand synthesis, and partial amplification. Sequencing libraries were prepared using the Nextera XT protocol and sequenced on an Illumina MiSeq instrument. The resulting sequence reads were filtered, reduced in complexity using unbiased de-novo assembly, and analysed for human viruses using BLAST. Only indubitable virus infections with more than 5 reads were counted in the downstream analyses. The association was assessed using conditional logistic regression methods.

Results: The number of good quality sequence reads ranged from 200,000 to 1.5 million per sample, most of which belonged to multiple bacteriophages and bacteria. At least one human virus was identified in 47% samples, with 13% samples being simultaneously positive for more than one human virus. We most often observed parechoviruses (35%), bocaviruses (13%) and sapoviruses (11%), whereas enteroviruses and rhinoviruses were rare. The overall coverage of the viruses ranged from several thousand-fold in a massively replicating sapovirus to sparsely distributed numbers of reads. We noted a clustering of virus findings within the matching groups, indicating adequate matching strategies. No globally significant virus association with the development of islet autoimmunity was observed at the genus level (all P values > 0.4).

Conclusion: This study shows a richness of human viruses previously not appreciated in the stools of otherwise healthy Finnish children, and underlines the utility of massive parallel sequencing of the virome. Further extension of this pilot dataset will allow studies of individual virus subtypes and strains, and also of sequence contigs not matching currently known organisms.

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Probiotic use in infancy and islet autoimmunity in the environmental determinants of diabetes in the young (TEDDY) study

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Background and aims: It is hypothesized that probiotics can prevent adverse immunological responses to environmental exposures by supporting the

healthy gut microbiota, and thus prevent the development to type 1 diabetes associated islet autoimmunity (IA) in young children. The aim of this study was to examine the association between early probiotic use and IA.

Materials and methods: The Environmental Determinants of Diabetes in the Young (TEDDY) study prospectively follows 8502 children with type 1 diabetes (T1D)-associated HLA-DR-DQ alleles in Finland, Germany, Sweden and the US. Blood samples were collected every 3 months from birth to evaluate the primary outcome of IA, defined as appearance of one or more of the islet autoantibodies GADA, IAA, or IA-2A confirmed at two consecutive visits. The introduction of either probiotic supplement or infant formula containing probiotics was classified as: early introduction (at the age of <3 months), or late or no introduction (at the age of 3 months and later, or not introduced during the first 12 months). We applied time-to-event analysis to study the association between probiotic use and IA, stratifying by country and adjusting for family T1D status, HLA type, sex, exclusive breastfeeding, and mode of delivery.

Results: Probiotic supplementation during the first 3 months either through dietary supplements and/or infant formula was most common in Finland (35.9%). In Germany 24.0%, in Sweden 11.6% and in the US 2.2% of children were given probiotics before the age of 3 months. Probiotic supplementation before 3 months of age was associated with a decreased risk of IA, compared with probiotic supplementation after 3 months or not at all (HR 0.62; 95% CI 0.45–0.84; $p=0.0018$). The country-specific hazard risk ratios for associations between the early probiotic supplementation and IA were: Finland 0.72 (95% CI 0.49–1.07; $p=0.10$), Germany 0.65 (95% CI 0.26–2.55; $p=0.37$), Sweden 0.42 (95% CI 0.21–0.85; $p=0.0165$), and the US 0.62 (95% CI 0.15–2.50; $p=0.50$).

Conclusion: Early probiotic supplementation may reduce the risk of IA in children who are at elevated genetic risk of T1D.

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Diabetic family history and beta cell autoimmunity in children with HLA-associated disease risk

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Background and aims: We assessed the role of a positive family history for type 1 diabetes (T1D) in relation to beta-cell autoimmunity in children carrying HLA-conferred disease risk, recruited from the general population.

Materials and methods: Children ($n=7410$, 52.7% males) with ($n=189$, 2.6%) and without ($n=7233$) T1D-affected first degree relatives (FDRs) were recruited as newborn infants from the general population, based on HLA-defined disease risk. They were observed for T1D-associated autoimmunity and progression to T1D for 13.6 years (median; range 0.9–18.6 years). In 1994–2002, the ICA assay was used for the primary autoantibody screening, after which IAA, GADA, and IA-2A were measured in seroconverted subjects, but since 2003, all four autoantibodies have been analyzed from all samples available.

Results: Children with T1D-affected FDRs had a higher frequency of seroconversions and multipositivity, higher levels of IAA and GADA, and progressed more often to T1D than children with no affected FDRs. The age at diagnosis was similar regardless of family background. Compared to children with no affected FDRs, the highest T1D risk was associated with paternal T1D (OR=11.9; $p<0.001$), followed by maternal (OR=3.5; $p<0.001$) and sibling-associated T1D (OR=3.4; $p=0.083$; sib vs. maternal T1D, $p=NS$). Children with T1D-affected FDRs had higher frequency of the high risk HLA genotype (DQB1*02:0302; 30.2 vs. 21.0%, $p=0.002$) than those with no family history for T1D. In the analyses of T1D-free survival in subjects who had developed at least one T1D-associated autoantibody, the 10-year progression rate since seroconversion was 66.1% (CI 47.7–84.5%) in children with paternal T1D, 37.7% (CI 12.8–62.6%) in children with maternal T1D, and 18.2% (CI 0–41.0%) in children with siblings affected by T1D (paternal vs. maternal, $p=0.035$), while the rate was 15.1% (12.9–17.2%) in children with no family history for T1D (paternal vs. no FDRs, $p<0.001$, and maternal vs. no FDRs, $p=0.006$). In the Cox regression analysis on seroconverted subjects, multipositivity (HR=7.2, CI 5.3–9.8), a positive family history for T1D (HR=2.0, CI

1.3–3.1), high risk genotype (HR=1.8, CI 1.4–2.4), young age at seroconversion (HR=0.7, CI 0.7–0.8), and higher levels of IAA (RU; HR=1.014, CI 1.007–1.021), IA-2A (RU; HR=1.010, CI 1.004–1.017), and ICA (JDFU; HR=1.004, CI 1.002–1.006) remained independent predictors of T1D.

Conclusion: Children with T1D-affected family members carry a higher risk for the initiation of beta-cell autoimmunity than their peers with no family history for T1D, even among children with moderate to high HLA-associated disease risk. Paternal T1D is an indicator of high risk for progression towards T1D, although the association between the high-risk HLA genotype and paternal T1D may partly explain the higher proportion of T1D observed in the offspring of affected fathers.

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A novel syndrome of early-onset type 1 diabetes and multi-organ autoimmunity caused by activating germline mutations in STAT3

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Background and aims: Type 1 diabetes (T1D) can be a feature of the monogenic polyautoimmune disorders, Immunodysregulation, Polyendocrinopathy, Enteropathy X-linked syndrome (IPEX) and Autoimmune Polyendocrinopathy Syndrome 1 (APS1). In these conditions autoimmune disease presents very early and in IPEX syndrome T1D often occurs ≤ 3 months. Investigation of individuals with early onset T1D associated with multiple early-onset autoimmune features may therefore reveal novel monogenic causes of autoimmunity.

Materials and methods: We studied patients with early onset Type 1 diabetes (<12 months) and early-onset autoimmune disease (<5 years). Initial investigation was looking for a de novo mutation by sequencing the exome of an individual with early onset T1D, primary hypothyroidism and coeliac disease and the unaffected parents. This was followed by further testing of 24 individuals with ≥ 2 autoimmune disorders (diagnosed <5 years) and 64 subjects with isolated diabetes diagnosed <6 months who are aged <5 years.

Results: The only de novo coding pathogenic variant was, p.T716M, in STAT3 in the patient with T1D, primary hypothyroidism and coeliac disease. Sanger sequencing identified a further 4 patients (3 polyautoimmune disease, 1 PNDM) with de novo STAT3 mutations (p.K392R, p.N646K (x2), p.K658N). All substitutions affect highly conserved residues and functional studies confirmed that all mutations increased STAT3 activity in vitro. Diabetes was present in 4/5 patients, presented early (2.5 [0–43] weeks (median [range])) and was insulin treated from diagnosis. Islet autoantibodies were detected in 3/4 patients supporting a diagnosis of autoimmune-mediated T1D. Additional autoimmune conditions included enteropathy, interstitial lung disease, juvenile-onset arthritis and primary hypothyroidism. Other common features were short stature (5/5 (<2SDS)) and eczema (4/5). The young age at presentation is consistent with STAT3 mutations causing accelerated autoimmune disease.

Conclusion: We report germline activating STAT3 mutations are a novel cause of early onset polyautoimmunity in which T1D is a common feature. This result confirms the crucial role of STAT3 in the pathophysiology of T1D. Whilst monogenic polyautoimmune disease is rare, these conditions are important as they offer valuable insights into the biology of the immune system in T1D.

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Family history of type 1 and type 2 diabetes and the risk of LADA—results from a population-based study of incident cases

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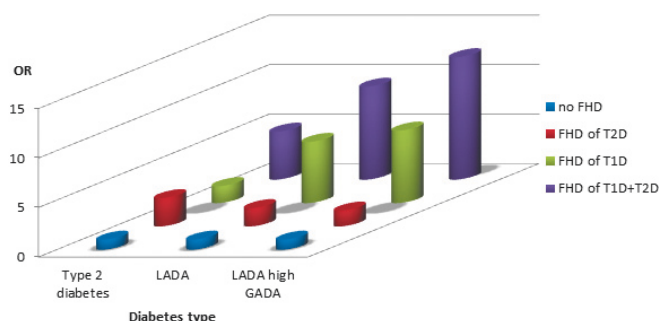
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Background and aims: LADA (latent autoimmune diabetes in adults) is suggested to be genetic mix of type 1 and type 2 diabetes, linked to HLA-DQB1 risk genotypes associated with autoimmunity as well as genes associated with type 2 diabetes, including *TCF7L2*. Family history of diabetes (FHD) encompasses both genetic and shared environmental factors and is a strong predictor of diabetes risk, although scarcely investigated in relation to LADA. Our aim was to investigate the risk of LADA in relation to family history of type 1 and type 2 diabetes.

Materials and methods: We used data from a population based case-control study with incident cases of adult onset (≥ 35 years) diabetes, including 264 cases of LADA (Glutamic acid decarboxylase antibodies (GADA) positive (>10 IU/mL) with c-peptide >0.3 nmol/l, 796 cases of type 2 diabetes (GADA negative), together with 1047 controls without diabetes, randomly selected from the population. Self-reported information on diabetes in first and second degree relatives was collected and relatives with onset <40 years and insulin treatment were classified as having type 1 diabetes (T1D) and otherwise as having type 2 diabetes (T2D). We estimated the odds ratio (OR) of LADA and T2D in relation to FHD, adjusted for age, sex and BMI.

Results: Family history of T1D (OR 6.2; 95% CI 1.8–20.8), and T2D (1.8; 95% CI 1.35–2.46) was associated with an increased risk of LADA and there were indications of a synergistically increased risk in those with a combination of T1D and T2D in the family (OR 9.39; 95% CI; 4.4–20.0). The association with FHD was even more pronounced for LADA with high GADA concentrations ($>$ median); OR was estimated at 7.3 (95% CI 1.91–28.0) for FHD of T1D and at 12.4 (95% CI 5.5–27.9) for the combination of T1D and T2D in the family. No difference was seen for FHD of T1D in female (OR 4.0; 95% CI 1.7–9.1) vs. male relatives (OR 4.4; 95% CI 1.9–10.2). The risk of T2D was associated with FHD of T2D (OR 2.9; 95% CI 2.3–3.6) but not FHD of T1D. In LADA patients, FHD of T1D vs T2D was associated with higher GADA (197.8 vs 139.5 IU/mL, $p=0.003$) but lower c-peptide (0.55 vs 0.81 nmol/l, $p=0.009$) levels.

Conclusion: The current data add support to the view that LADA is an admixture of both T1D and T2D, also in terms of genetic risk.



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Low birth weight is associated with an increased risk of latent autoimmune diabetes in adults (LADA) and type 2 diabetes: results from ESTRID a Swedish case-control study

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Background and aims: A large body of research has recognized low birth weight as a risk factor for type 2 diabetes (T2D), hypothetically as an indicator of poor nutrition of the fetus, leading to insulin resistance. Genetic factors have also been implicated, e.g. it has been suggested that effects of paternal diabetes may be mediated by effects on intrauterine environment, manifested in low birth weight of the offspring. In contrast a link between high birth weight and type 1 diabetes (T1D) has been documented. Our aim was to investigate, for the first time, the association between birth weight and LADA, a common diabetes form with features of both T1D and T2D.

Materials and methods: We used data from ESTRID (Epidemiological Study of Risk factors for LADA and type 2 Diabetes), a Swedish population-based study. Eligible for the analysis were 116 incident LADA cases (\geq age 35 and Glutamic Acid Decarboxylase Antibodies (GADA) positive (>10 IU/mL) with c-peptide >0.3 nmol/l), 298 incident T2D cases (\geq age 35 and GADA negative) and 521 disease-free controls randomly sampled from the population. Information on birth weight and covariates was based on self-report. We present Odds Ratios (OR) and 95% Confidence Intervals (CI) calculated by logistic regression and adjusted for sex, age, current BMI (kg/m^2) and family history of diabetes (FHD) in first and second degree relatives.

Results: Low birth weight was associated with an increased risk for LADA as well as T2D; OR per kilogram reduction was estimated at 1.40 (CI; 1.03–1.90) and 1.39 (CI; 1.07–1.81) respectively and OR for subjects weighing less than 3 kg compared to more than 4 kg at birth was estimated at 2.06 (CI; 1.07–3.97) for LADA and 1.98 (CI; 1.16–3.40) for T2D. Further adjustments for education, physical activity, smoking and alcohol did not affect these estimates. Indications of an excess risk was seen both in those with FHD (OR 1.24, CI; 0.79–1.95) and without FHD (OR 1.59, CI; 1.04–2.43). A combination of low birth weight (<3 kg) and current obesity (BMI ≥ 30) further augmented the risk; OR 3.69, CI; 1.33–10.26 (LADA) and 14.72, CI; 6.44–33.62 (T2D).

Conclusion: Our results suggest that low birth may also be a risk factor for LADA of the same strength as for T2D. The association appeared to be independent of family history of diabetes, which provides some support for the hypothesis that environmental rather than genetic factors explain this link. The combination of low birth weight and adult obesity appears to be a particularly strong risk factor, this being in line with the thrifty phenotype hypothesis.

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OP 30 Integrative physiology

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A novel mitochondrial mechanism controlling insulin secretion in obesity

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Background and aims: Reactive oxygen species can increase or decrease insulin secretion. However, it is unknown whether inhibiting an antioxidant system in islets can lead to improved insulin secretion without affecting their viability. Our goal is to identify whether modulating components related to heme metabolism, known to increase reactive oxygen species production but also antioxidant activity, can increase secretion without affecting viability. To this end, we studied the role of the mitochondrial transporter ATP-binding cassette B10 (ABCB10) regulating glucose stimulated insulin secretion in the context of diet-induced obesity, as ABCB10 is essential for proper heme metabolism and protection from oxidative stress.

Materials and methods: Obesity in mice was induced by high fat diet feeding (45% Fat, Research Diets). Mice fed hypercaloric control diet (CD, calories matched with carbohydrates) or chow diet were used as controls. Glucose tolerance tests (1g/kg I.P.) after fasting were performed and both insulin (ELISA, ALPCO) and glucose (FreeLite glucometer) were measured. Studies were carried out along BU IACUC and “Principles of laboratory animal care” (NIH). Mouse models are whole body ABCB10 +/- mice (n=10 backcrossed) and ABCB10-LoxP+/+ mice (pure) in C57Bl6J background. Islets were isolated 14–24 weeks after diet onset (weaning). Glucose stimulated insulin secretion was performed in modified DMEM by incubating islets in 2.8 mM glucose or 16.7 mM glucose for 30 minutes. Insulin in the media was measured by FRET (HTRF, CisBio). In the case of ABCB10-LoxP islets, they were transduced with Cre or Ds-Red control adenovirus at MOI 200 after isolation. Insulin was measured as in ABCB10 +/- islets. ABCB10 Real time PCR was performed using TaqMan probes.

Results: High fat diet and control diet feeding for 5 months had the expected effects increasing weight, fasting glucose and insulin levels, when compared to chow diet feeding. No significant differences in these parameters were detected between wild type and ABCB10 +/- mice though. However, glucose tolerance tests showed that ABCB10 +/- mice were more tolerant than wild type mice and only after high fat diet feeding. This increase in glucose tolerance was associated with higher blood insulin levels in response to glucose. In this regard, only high fat diet feeding resulted in ABCB10 +/- islets showing higher glucose stimulated insulin secretion when compared to wild type (40% increase). To address whether ABCB10 acutely regulated insulin secretion, we inactivated ABCB10 alleles after high fat diet feeding by Cre adenoviral expression in ABCB10 LoxP+/+ or LoxP+/- islets. Using this approach, we reduced ABCB10 expression by 80–90% and 40–50% respectively. We found that only after high fat feeding, glucose stimulated insulin secretion was increased in ABCB10 knock out (LoxP+/+ - Cre; ~30%) and in LoxP+/- Cre (~15%), when compared to respective DsRed transduced controls. This increase in insulin secretion was also present in ABCB10 knock out islets from chow diet mice just exposed for 24 hours to high glucose (20mM) and NEFA (palmitate 0.4 mM).

Conclusion: We report an unexpected role for the mitochondrial transporter ABCB10 regulating insulin secretion specifically in the context of high fat diet. We propose that lack of ABCB10 increases a reactive oxygen species-related high fat specific signal in islets that stimulates insulin secretion. In all, ABCB10 modulation could be a potential approach to improve insulin secretion specifically in the context of obesity.

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Circulating irisin is up-regulated by insulin infusion in obese, but not in lean humans, and is inversely associated with insulin sensitivity and respiratory exchange ratio

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Background and aims: Irisin is a recently discovered myokine, with the potential to induce brown-fat-like development of white adipose tissue and to increase energy expenditure. In humans, data on the relationships of circulating irisin with insulin sensitivity and other metabolic parameters are inconsistent. The aim of the present study was to assess the associations of serum irisin with insulin sensitivity and substrate oxidation, as well as the effect of insulin infusion on circulating irisin.

Materials and methods: The study group consisted of 148 healthy subjects (115 males and 33 females, age between 18 and 35 years), 81 lean and 67 overweight or obese. Euglycemic hyperinsulinemic clamp and indirect calorimetry were performed. Serum irisin was measured in the baseline state and after the clamp.

Results: Baseline irisin was not different between lean and overweight/obese group ($p=0.44$). Insulin infusion resulted in an increase in serum irisin in overweight/obese ($p=0.006$), but not in the lean group ($p=0.73$). In consequence, post-clamp irisin was higher in the overweight/obese ($p=0.042$). Baseline irisin was related to fasting NEFAs ($r=0.26$, $p=0.008$). Post-clamp irisin was positively related to BMI, waist circumference, triglycerides and resting energy expenditure (all $p<0.05$) and negatively to insulin sensitivity ($r=-0.20$, $p=0.014$) and respiratory exchange ratio, both at baseline ($r=-0.21$, $p=0.046$) and during the clamp ($r=-0.34$, $p=0.001$). Concordantly with the latter finding, we observed negative association of post-clamp irisin with glucose oxidation ($r=-0.26$, $p=0.008$) and positive with lipid oxidation ($r=0.37$, $p<0.0001$) during hyperinsulinemia.

Conclusion: Our data show that insulin increases circulating irisin, but this effect is dependent on the presence of overweight/obesity and may represent a compensatory response to increase lipid oxidation in these conditions.

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Maternal BMI and exercise have interactive effects on brain's white and grey matter composition in elderly women

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Background and aims: Maternal obesity during pregnancy is a major health risk for the offspring and it may have a permanent effect on the offspring's subsequent morbidity. Cardiovascular diseases and type 2 diabetes share common early life risk factors and start from early age. Frailty is considered a precursor of ageing. Exercise training programs promote beneficial effects on health and can be effective even in elderly subjects. Nevertheless, no data is available on brain morphometric changes in frail subjects, and the effects of exercise on brain tissue composition. The object of the study was to measure the possible differences in grey (GM) and white matter (WM) density between frail offspring of obese (OOM) and lean mothers (OLM). We hypothesized that i) OOM have lower WM density compared to OLM and that ii) the OLM subjects will have higher capacity to increase WM density compared to OOM following the exercise intervention.

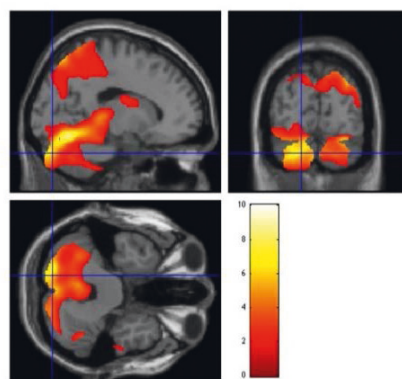
Materials and methods: 36 frail women aged 68–79 years were studied at baseline and 27 of them after four months of resistance exercise training. The participants were recruited from a large cohort from the Helsinki Birth Cohort Study (HBCS) according to the body mass index of their mothers when giving birth (16 from highest quartile and 20 from the two lowest quartiles). T1-weighted MRI images were segmented into GM and WM using SPM and

VBM8 toolbox, and compared using *t* tests. Data were thresholded at $p < 0.05$, FDR corrected at cluster level.

Results: WM but not GM density was higher in OLM versus OOM globally in the brain. Exercise intervention increased WM density in both OLM and OOM subjects in the cerebellum. The intervention increased WM and decreased GM density in parietal superior regions in OLM and in cuneus and precuneus regions in OOM. When comparing OLM and OOM for the changes in WM density following intervention, cerebellum of the OLM showed higher increase of WM density compared to OOM (Figure 1).

Conclusion: Maternal BMI during pregnancy influences brain atrophy in their offspring, especially the white matter. We show that exercise has an impact on brain morphology even in elderly women and in a brief time period. Being born to an obese mother compared to a lean or normal weight mother implies lower brain adaptability to exercise-induced changes in the WM density. These changes were more pronounced in the cerebellum region and this could have been caused by axon caliber and myelination increase in a similar way to what happens in subjects learning a new motor skill.

Figure 1. Exercise intervention differences (OLM higher than OOM) in White Matter Density



Clinical Trial Registration Number: NCT01931540

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Plasma FGF21 is released from the splanchnic circulation in response to exercise and regulated by insulin and glucagon: a human study

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Background and aims: FGF21 is a circulating protein that has beneficial metabolic effects. An increase in plasma FGF21 has been observed with fasting and exercise; probably secreted from the liver. However the regulation of plasma FGF21 in normal physiology is not completely understood. Aims: 1) to demonstrate that FGF21 is released from the liver in response to an acute exercise bout. 2) to investigate if glucagon and/or insulin regulates plasma FGF21 levels.

Materials and methods: A: Ten healthy young males performed a 2 hour exercise bout, with a hepatic vein and arterial catheter allowing drawing blood repeatedly and simultaneously before, during and after a 2 hour bicycling exercise bout at 60% VO_2max . Hepatic blood flow was determined by use of indocyanine green. B: Ten subjects underwent 4 trails resting in supine position: 1) 1 hour of glucagon infusion 2) 1 hour of glucagon combined with a co-infusion of somatostatin 3) 1 hour of somatostatin infusion, 4) 1 hour of saline infusion (control). Blood samples were obtained every hour during the 8 hour experimental day. Plasma insulin, glucagon and FGF21 was measured by immunoassays.

Results: The exercise study with the arterial-hepatic venous difference demonstrated a higher level of insulin and glucagon in the hepatic vein compared to the artery. During the exercise bout a decrease in plasma insulin and an increase in plasma glucagon were observed, which normalised in the recovery after the bicycling exercise. Plasma FGF21 gradually increased during the exercise bout to a 5-fold increase and rapidly returned to basal level into recovery. Calculating the arterial- hepatic vein differences revealed a net release of FGF21 at rest before the bicycling commenced. The secretion of FGF21 increased gradually during the 2 hours of bicycling and returned to baseline in the recovery. The hepatic blood flow did not change during the experiment.

In infusion study 1 (glucagon alone), plasma glucagon increased during the 1 hour infusion followed by compensatory increase in plasma insulin. In trial 2 (glucagon + somatostatin) glucagon increased similarly to trial 1, with no compensatory insulin increase. In trial 3 (somatostatin alone) both insulin and glucagon decreased, compared to trial 4 (saline), where no changes were observed. Interestingly, plasma FGF21 increased with a similar kinetics as observed with exercise, only when glucagon was infused and the compensatory insulin response was blocked by somatostatin. A delayed increase in plasma FGF21 was observed in trial 3 (somatostatin alone).

Conclusion: Here we present the first human data demonstrating by a direct measurement across the splanchnic organs that FGF21 is released. The liver is the most likely organ to be responsible; however contributions from other organs as the spleen, intestines or pancreas cannot be excluded. Furthermore our data demonstrate that the splanchnic circulation releases exercise-induced FGF21 in humans. Exercise increased plasma glucagon concomitantly with a decrease in insulin and simulating this condition in resting healthy men, increased plasma FGF21 with a similar kinetics as observed with exercise. These data add to the understanding of FGF21 in human physiology.

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Selective expression of ROCK1 in the liver promotes insulin resistance and hepatic steatosis in diet-induced obese mice

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Background and aims: In the European Union approximately 29 million people suffer from a chronic liver condition. The prevalence of non-alcoholic fatty liver disease (NAFLD) is 2-44% in the European population and 42.6-69.5% in people with type 2 diabetes. Furthermore, over 50% of adults in the European Union are overweight or obese. Obesity is a risk factor for NAFLD and is strongly associated with insulin resistance. Our previous data showed that liver-specific deletion of Rho-kinase 1 (ROCK1) caused a significant improvement in insulin sensitivity and hepatosteatosis in obese mice induced by a high-fat diet. The current study was designed to further determine the physiological role of hepatic ROCK1 in regulating whole-body glucose and lipid metabolism.

Materials and methods: Mice expressing a constitutively active (CA) mutant of ROCK1 in liver were studied. These mice started a high-fat diet (HFD) at 6 weeks of age for a period of 12 weeks. Insulin sensitivity and glucose tolerance were assessed and body weight and glucose levels were also monitored. Hepatic and serum content in triglycerides and cholesterol was determined. Hematoxylin and eosin stain (H&E stain) of liver sections from control and CA-ROCK1 mice was performed. Gene expression of key molecules involved in lipid metabolism was also determined for control and CA-ROCK1 mice.

Results: Liver-specific CA-ROCK1 mutant mice exhibited higher body weight 2 weeks after HFD feeding (21.6 ± 0.4 g N=10 vs. 23.6 ± 0.6 g N=10, $p = 0.01$) and this difference was increased by the period on HFD (33.8 ± 1.28 g N=10 vs. 39.4 ± 1.4 g N=10, $p = 0.01$). Blood glucose was also increased after 4 weeks of HFD (148.0 ± 3.7 mg/dL N=10 vs. 165.4 ± 7.1 mg/dL N=10, $p = 0.05$). These mice were insulin resistant, as revealed by the failure of blood glucose levels to decrease after insulin injection (AUC 16058 ± 594.0 N=6 vs. 20581 ± 1102 N=10, $p = 0.01$), but normal glucose tolerant. These effects were accompanied by hyperinsulinemia, increased hepatic triglycerides (430.2 ± 40.7 mg/dL N=9 vs. 674.1 ± 108.0 mg/dL N=8, $p = 0.05$) and serum cholesterol (94.2 ± 8.4 mg/dL N=10 vs. 131.4 ± 12.6 mg/dL N=9, $p = 0.05$) in the CA-ROCK1 mice. Histological analysis showed that hepatic steatosis by high-fat feeding was greatly increased in liver-specific CA-ROCK1 mutant mice compared with control mice. Moreover, activation of ROCK1 in liver caused an increase in gene expressions of key lipogenic enzymes, including FAS (fatty acid synthase) and ACC (Acetyl-CoA carboxylase). However, overexpression of hepatic CA-ROCK1 had no effect on gene expression involved in fatty acid oxidation and fatty acid uptake.

Conclusion: Our data demonstrate that activation of hepatic ROCK1 is sufficient to cause insulin resistance and hepatic steatosis in diet-induced obese mice, suggesting an important role for hepatic ROCK1 in regulating fuel me-

tabolism. Thus, hepatic ROCK1 could be a molecular target for the treatment of obesity and obesity-related metabolic disorders such as NAFLD.

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Prediabetes and type 2 diabetes are associated with increased content of dipeptide carnosine in human skeletal muscle

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Background and aims: Carnosine (β -alanine L-histidine) is dipeptide commonly found in mammalian tissues and enriched in skeletal muscle. It has been shown to suppress many biochemical processes that accompany ageing and age related chronic diseases. Recent animal studies suggested the importance of carnosine supplementation for glycemic control and prevention of type 2 diabetes. Our aim was to analyze skeletal muscle carnosine content in middle aged (45.1 ± 1.2 years) lean healthy (BMI 24.5 ± 0.4 kg.m⁻²), overweight/obese (BMI 29.3 ± 0.8 kg.m⁻²), prediabetic (BMI 32.1 ± 0.7 kg.m⁻²) and type 2 diabetic (BMI 30.7 ± 0.9 kg.m⁻²) individuals (n=9/group).

Materials and methods: Metabolic phenotyping included euglycemic hyperinsulinemic clamp (insulin sensitivity), oral glucose tolerance test, MRI (abdominal adipose tissue content & distribution) and indirect calorimetry (resting energy expenditure). Physical activity was monitored with accelerometers and validated questionnaire. Samples of *m.vastus lateralis* were obtained by needle biopsy. Skeletal muscle carnosine content (HPLC) and serum CN1 carnosinase activity were determined. Mitochondrial biogenesis related genes as well as relative muscle mitochondrial content were assessed by qPCR.

Results: Skeletal muscle carnosine content progressively increased in patients with prediabetes (by 30%, NS) and type 2 diabetes (by 39%, $p < 0.05$) as compared to lean individuals. It was positively associated with BMI ($R = 0.44$, $p = 0.007$), % body fat ($R = 0.35$, $p = 0.037$), abdominal subcutaneous adipose tissue content ($R = 0.38$, $p = 0.024$) as well as with atherogenic index ($R = 0.35$, $p = 0.04$), fasting and 2h glycaemia ($R = 0.39$, $p = 0.02$; $R = 0.55$, $p = 0.002$). Muscle carnosine content was inversely associated with insulin sensitivity ($R = -0.42$, $p = 0.006$), percentage of high and moderate intensity ambulatory activity ($R = -0.42$, $p = 0.011$) as well as with resting energy expenditure ($R = -0.58$, $p < 0.001$). Associations of muscle carnosine with 2h glycaemia and resting energy expenditure were at least in part independent on BMI. Muscle carnosine levels were not related to mitochondrial content and *Pgc1 α* and *cytochrome C* mRNA. Serum CN1 carnosinase activity was not regulated in obesity and T2D.

Conclusion: We observed increased skeletal muscle carnosine content in patients with type 2 diabetes. This might represent a potential adaptive mechanism counteracting disturbances related to diabetic status.

Clinical Trial Registration Number: NCT02011100

Supported by: VEGA 2/0192/14

OP 31 GLP-1 analogues: non-glycaemic endpoints

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Effect of liraglutide 3.0/1.8 mg on body weight and cardiometabolic risk factors in overweight/obese adults with type 2 diabetes: SCALE diabetes randomised, double-blind, 56-week trial

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Background and aims: Obesity increases the risk of multiple comorbidities including type 2 diabetes mellitus (T2D) and cardiovascular (CV) disease. Weight loss of 5–10% can decrease the risk, but is difficult to achieve and maintain by lifestyle changes alone. Liraglutide at doses up to 1.8 mg is approved for treatment of T2D and is associated with weight loss in addition to glycaemic control. This study evaluated the effects of liraglutide (3.0 and 1.8 mg) as adjunct to diet and exercise on weight loss (primary endpoint) and cardiometabolic risk factors (waist circumference, glycaemia, blood pressure [BP], pulse, fasting lipids, and CV biomarkers) in overweight/obese adults with T2D.

Materials and methods: Subjects were randomised 2:1:1 to liraglutide 3.0 mg, 1.8 mg, or placebo for 56 weeks; all arms included a 500 kcal/day deficit diet and exercise.

Results: A total of 846 individuals (male 50%, mean age 54.9 years, BMI 37.1 kg/m² [27.0–67.6], HbA1c 7.9%, diabetes duration 7.3 years [0.2–36.5], 11.5% on diet and exercise, 57.3% on metformin alone, and 31.2% on combination OADs) were randomised into the trial. At week 56, least square (LS) mean weight loss was 5.9%, 4.6% and 2.0% for 3.0 mg, 1.8 mg, and placebo, respectively; 3.0 mg and 1.8 mg were superior to placebo ($p < 0.0001$); 3.0 mg was superior to 1.8 mg ($p = 0.0024$). Weight loss was accompanied by reductions in waist circumference of 6.0, 4.9, and 2.8 cm respectively; 3.0 mg and 1.8 mg were superior to placebo ($p \leq 0.0004$); 3.0 mg was superior to 1.8 mg ($p = 0.0224$). HbA1c decreased by 1.32%, 1.13% and 0.38% from baseline with 3.0 mg, 1.8 mg, and placebo respectively ($p < 0.0001$ vs. placebo; $p = 0.0125$ for 3.0 mg vs. 1.8 mg). At baseline, systolic (S) /diastolic (D) BP and lipids were well controlled. SBP reductions were 3.0, 3.1, and 0.4 mmHg from baseline (~ 130 mmHg) for the 3 arms respectively; greater with 3.0 mg and 1.8 mg ($p < 0.05$ vs. placebo), with no significant differences between treatments in DBP at week 56. Similar LS mean pulse increases of 2.0 and 2.2 beats/min occurred with liraglutide 3.0 and 1.8 mg vs. -1.5 beats/min for placebo ($p < 0.0001$ vs. placebo) (observed means, LOCF: 2.0 and 2.1 vs. -1.4 beats/min, respectively). Liraglutide 3.0 mg reduced total cholesterol, VLDL and triglycerides (4%, 13%, 14%, $p < 0.05$) and increased HDL (+3%, $p < 0.05$) vs. placebo whereas 1.8 mg had no effects. Liraglutide had no significant effects on LDL and FFA. Reductions in high-sensitivity C reactive protein occurred with liraglutide 3.0 and 1.8 mg (-27% and -25% vs. placebo, $p \leq 0.0002$). Decreases in plasminogen activator inhibitor-1 (-24%, $p = 0.0004$) and urinary albumin/creatinine ratio (-20%, $p = 0.0086$) occurred only with liraglutide 3.0 mg vs. placebo; No treatment effects on adiponectin occurred. Fibrinogen increased by 5% vs. placebo with 3.0 mg only ($p = 0.046$).

Conclusion: After 56 weeks' treatment, liraglutide 3.0 mg, as an adjunct to diet and exercise, caused significant weight loss that was associated with additional benefits for multiple cardiometabolic risk factors compared to liraglutide 1.8 mg and placebo in overweight/obese adults with T2D.

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Efficacy and safety of liraglutide versus placebo in subjects with type 2 diabetes and moderate renal impairment (LIRA-RENAL): a randomised trial

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Background and aims: Renal impairment in type 2 diabetes mellitus (T2DM) limits the available glucose lowering treatment options. The rationale for this trial was to establish the efficacy and safety of liraglutide 1.8 mg as add-on to existing glucose lowering drugs and/or insulin therapy in subjects with inadequately controlled T2DM and moderate renal impairment (estimated glomerular filtration rate [eGFR] 30–59 mL/min/1.73 m²; MDRD).

Materials and methods: In this 26-week, double-blind, multicentre, multinational, parallel group trial, adults with T2DM and moderate renal impairment, BMI of 20–45 kg/m², HbA1c of 7.0–10.0% and on stable diabetes medication (unchanged medication and dose for ≥90 days prior to screening) were randomised 1:1 to receive either once-daily liraglutide 1.8 mg or placebo. Liraglutide was initiated at 0.6 mg/day and incremented by 0.6 mg/day on a weekly basis to the target dose of 1.8 mg/day. The primary endpoint was change in HbA1c from baseline (BL) to Week 26 (Table).

Results: 279 subjects were randomised (140 to liraglutide; 139 to placebo). All subjects, except for 2 in the placebo group, were exposed to trial medication and included in the analysis. Liraglutide 1.8 mg showed superior glycaemic control relative to placebo in subjects with moderate renal impairment with a low risk of hypoglycaemia and reduced body weight (Table). The most common adverse events (AEs) were GI side effects (liraglutide 35.7%, placebo 17.5%), mostly nausea and vomiting which resolved quickly. There was a higher incidence of AE leading to withdrawals in the liraglutide group (13.6%), compared to placebo (2.9%). No deterioration in renal function was observed (eGFR change from BL: -1% liraglutide; +1% placebo $p=0.36$). There was an increase from baseline in amylase and lipase levels in the liraglutide group that was not seen in the placebo group. One subject with elevated lipase (>3x ULN) and amylase (>2x ULN) at baseline was diagnosed with chronic pancreatitis on Day 11 of treatment with liraglutide.

Conclusion: Liraglutide 1.8 mg showed superior HbA1c and weight reduction with no unexpected safety or tolerability issues including no worsening of renal function in subjects with moderate renal impairment over 26 weeks. The efficacy, low incidence of hypoglycaemia and safety of liraglutide in subjects with T2DM and moderate renal impairment was demonstrated.

BASELINE	Liraglutide 1.8 mg (N=140)	Placebo (N=137)	ENDPOINT Week 26	Liraglutide 1.8 mg	Placebo	Treatment difference or ratio (95% CI; p-value)
Age, years, mean (SD)	68.0 (8.3)	66.3 (8.0)	HbA _{1c} , % change from BL, mean	-1.05	-0.38	-0.66 (-0.90; -0.43; $p<0.0001$)
Duration of diabetes, years, mean (SD)	15.86 (8.86)	14.17 (7.52)	HbA _{1c} <7%, proportions and odds ratio	52.8%	19.5%	4.64 (2.54; 8.46; $p<0.0001$)
No insulin ± OAD, %	45.0	44.5	HbA _{1c} <7% and no weight gain, proportions and odds ratio	46.0%	16.0%	4.48 (2.46; 8.18; $p<0.0001$)
Basal insulin ± OAD, %	20.7	17.5	HbA _{1c} <7%, no weight gain and no minor/severe hypoglycaemia, proportions and odds ratio	27.9%	8.5%	4.14 (2.20; 7.80; $p<0.0001$)
Premix insulin ± OAD, %	34.3	38.0	Hypoglycaemia, proportions and odds ratio			0.50 (0.23; 1.08; $p=0.0760$)
HbA _{1c} , % mean (SD)	8.08 (0.79)	8.00 (0.85)	Body weight, kg, change from BL, mean	-2.41	-1.09	-1.32 (-2.24; -0.40; $p=0.0052$)
eGFR (MDRD) mean (SD) (mL/min/1.73 m ²)	46.6 (10.3)	46.9 (11.7)	Albumin:creatinine ratio to BL, mean	0.87	1.05	0.83 (0.62; 1.10; $p=0.1856$)
Body weight, kg, mean (SD)	93.6 (17.4)	95.6 (17.7)	Subjects with amylase >ULN (reference range 28.0–100.0 U/L)	21 (20.0%)	22 (20.2%)	N/A*
BMI, kg/m ² , mean (SD)	33.4 (5.4)	34.5 (5.4)	Subjects with lipase >ULN (reference range 16.0–63.0 U/L)	51 (48.6%)	22 (20.4%)	N/A*
Albumin:creatinine ratio (mg/mmol) (SD)	44.3 (96.3)	41.8 (93.7)				
Subjects with amylase >ULN (reference range 28.0–100.0 U/L)	18 (12.9%)	23 (16.8%)				
Subjects with lipase >ULN (reference range 16.0–63.0 U/L)	44 (31.4%)	33 (24.1%)				

*Analysed by descriptive statistics; BL=baseline; eGFR=estimated glomerular filtration rate; MDRD=modification of diet in renal disease; OAD=oral antidiabetic drug; SD=standard deviation; ULN=upper limit of normal range

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Supported by: Novo Nordisk

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Effect of saxagliptin on renal outcome

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Background and aims: It was previously suggested that DPP-4 inhibitors may have a protective effect on diabetic kidney disease. Herein we studied the renal outcomes of 16,492 patients (pts) with type 2 diabetes, who were prospectively followed in the SAVOR TIMI 53 trial for a median of 2.1 years. Predefined renal outcomes (safety and efficacy) were: doubling of serum creatinine, the composite of initiation of chronic dialysis, renal transplant or serum creatinine >6.0 mg/dl, reduction from baseline of albumin/creatinine ratio (ACR) and categorical changes in ACR.

Materials and methods: At baseline, pts were stratified into 3 groups according to renal function: normal or mild renal dysfunction (RD) [eGFR >50 mL/min; N=13,916], moderate RD [eGFR 30–50 mL/min; N=2,240], or severe RD [eGFR <30 mL/min; N=336]. Baseline categorical ACR was normal (ACR <30 mg/G) in 9,696 (58.8%) pts, microalbuminuria (ACR 30–300 mg/G) in 4,426 (26.8%) pts and macroalbuminuria (ACR >300 mg/G) in 1,638 (9.9%) pts.

Results: Patients assigned to saxagliptin (SAXA) vs. placebo (PLB) were more likely to improve their categorical ACR (11% vs. 9% $p<0.01$) and less likely to worsen their categorical ACR (13% vs. 16% $p<0.01$). In patients with microalbuminuria at baseline, treatment with saxagliptin was associated with greater reversal to normoalbuminuria: at 1 year, 31.3% vs. 25.7%, respectively ($p<0.0001$). These results were maintained for 2 years and at end of trial (EOT). At an “on treatment analysis”, the improvements in the SAXA group compared with the PLB group were observed in ACR and in all eGFR categories and at all time points (Table). Pearson correlation analysis showed that the change in albuminuria was independent of the change in HbA1c at 1 year, 2 years, and EOT (Pearson coefficients: 0.015, 0.025, and 0.033, respectively). There were no differences in the other predefined renal outcomes between the SAXA and PLB groups: doubling of serum creatinine [153 (0.92%) vs. 147 (0.89%), HR 1.04 (0.83–1.30)], the composite of initiation of chronic dialysis, renal transplant or serum creatinine >6.0 mg/dl [51 (0.31%) vs. 55 (0.33%), HR 0.90 (0.61–1.32)] or the composite end point of death and all of the above [573 (3.47%) vs. 525 (3.20%), HR 1.08 (0.96–1.22)].

Conclusion: In the SAVOR TIMI 53 trial, saxagliptin improved ACR and had a neutral effect on the other predefined renal outcomes. The beneficial effect of saxagliptin on albuminuria seems to be independent of its effect on glycaemia.

Mean Change from Baseline of ACR (mg/G) by eGFR Categories, Time Point and Treatment Groups (On-Treatment Analysis)

eGFR group N (at baseline)	Time point	Treatment group	N	Mean change in ACR from baseline (mg/G)	Difference in mean change in ACR between SAXA and Placebo (mg/G)	95% CI of difference in ACR between SAXA and Placebo (mg/G)	P values for difference in ACR between SAXA and Placebo
Total N=15760	1 year	Saxagliptin	6579	-6.5			
		Placebo	6424	26.1	-31.7	-49.6; -13.7	<0.001
	2 year	Saxagliptin	5732	31.9			
		Placebo	5542	64.9	-34.3	-53.4; -15.3	<0.001
eGFR >50 mL/min/BSA N=13319	1 year	Saxagliptin	6625	5.2			
		Placebo	5508	24.0	-18.9	-35.5; -2.3	0.026
	2 year	Saxagliptin	4935	35.9			
		Placebo	4774	53.9	-19.3	-37.0; -1.6	0.033
30 ≤ eGFR ≤ 50 mL/min/BSA N=2128	1 year	Saxagliptin	833	-54.3			
		Placebo	804	57.4	-81.4	-158.3; -4.5	0.038
	2 year	Saxagliptin	690	20.9			
		Placebo	678	115.8	-105.0	-186.4; -23.6	0.011
eGFR <30 mL/min/BSA N=313	1 year	Saxagliptin	121	-222.2			
		Placebo	112	48.3	-257.6	-523.3; 8.2	0.057
	2 year	Saxagliptin	107	-84.7			
		Placebo	90	294.3	-245.2	-525.1; 34.7	0.066

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Liraglutide 3.0 mg reduces severity of obstructive sleep apnoea and body weight in obese individuals with moderate or severe disease: SCALE sleep apnoea trial

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Background and aims: Obesity is strongly associated with obstructive sleep apnoea (OSA) and weight loss has been shown to reduce disease severity. This randomised, double-blind, parallel-group trial compared the effects of liraglutide 3.0 mg to placebo, both as adjunct to diet (500 kcal/day deficit) and exercise, on OSA severity and body weight. The primary endpoint was change in apnoea-hypopnoea index (AHI, number of apnoea/hypopnoea events per hour of sleep) after 32 weeks (Table).

Materials and methods: Obese individuals without diabetes who had moderate (AHI 15–29.9 events/h) or severe (AHI ≥30 events/h) OSA and were unwilling/unable to use continuous positive airway pressure therapy were randomised 1:1 to liraglutide 3.0 mg or placebo for 32 weeks. Of 359 randomised individuals (mean baseline age 48.5 years, males 71.9%, AHI 49.2 events/h, severe OSA 67.1%, body weight 117.6 kg, BMI 39.1 kg/m², HbA1c 5.7%), 276 (76.9%) completed the trial.

Results: After 32 weeks, the reduction in AHI was significantly greater with liraglutide 3.0 mg than with placebo (−12.2 vs. −6.1 events/h, $p=0.0150$) (Table). Supporting the reduction in AHI, endpoints related to oxygen saturation, polysomnographic measures of sleep quantity and efficiency, and quality of life also improved with liraglutide 3.0 mg, albeit statistically non-significantly compared to placebo. Liraglutide 3.0 mg produced significantly greater mean percentage weight loss compared to placebo (−5.7 vs. −1.6%, $p<0.0001$) and enabled more individuals to reach ≥5% and >10% weight loss targets after 32 weeks. Post-hoc analysis showed a relationship between weight loss and change in AHI. In addition, there were significantly greater reductions in neck circumference, HbA1c and systolic blood pressure (SBP) with liraglutide 3.0 mg versus placebo. After 32 weeks, heart rate increased by about 2 beats/min with liraglutide 3.0 mg compared to placebo. The safety profile of liraglutide 3.0 mg was generally consistent with that previously seen with liraglutide in type 2 diabetes. Nausea and diarrhoea were the most common adverse events with liraglutide 3.0 mg (27% and 17% of individuals, respectively); most events were mild/moderate and transient.

Conclusion: As an adjunct to diet and exercise, liraglutide 3.0 mg was generally well tolerated and produced significantly greater reductions than placebo in AHI, body weight, SBP and HbA1c in obese individuals with moderate/severe OSA. The results also indicate that weight loss improves OSA-related parameters.

Table. Effects of liraglutide 3.0 mg on parameters related to OSA, body weight, glycaemia and blood pressure after 32 weeks of treatment.

Change from baseline after 32 weeks	Liraglutide 3.0 mg <i>n</i> =180 LS mean (LOCF)	Placebo <i>n</i> =179 LS mean (LOCF)	Liraglutide 3.0 mg vs. Placebo estimated TD ¹ or OR ²
AHI ³ (events/h)	−12.2	−6.1	−6.1 ($p=0.0150$) ¹
Oxygen desaturation ≥4% index (events/h)	−9.5	−5.1	−4.4 ($p=0.0608$) ¹
Total sleep time (min)	23.2	15.5	7.7 ($p=0.1629$) ¹
Wake time after sleep onset (%)	−4.6	−2.9	−1.6 ($p=0.0994$) ¹
Body weight (%)	−5.7	−1.6	−4.2 ($p<0.0001$) ¹
≥5% body weight loss (%)	46.4	18.1	3.9 ($p<0.0001$) ¹
>10% body weight loss (%)	22.4	1.5	19.0 ($p<0.0001$) ¹
Neck circumference (cm)	−2.1	−1.3	−0.8 ($p=0.0014$) ¹
HbA _{1c} (%-point)	−0.4	−0.2	−0.2 ($p<0.0001$) ¹
SBP (mmHg)	−3.7	0.4	−4.1 ($p=0.0003$) ¹

AHI=apnoea-hypopnoea index, LOCF=last observation carried forward, LS=least square, *n*=number of randomised subjects, OSA=obstructive sleep apnoea, SBP=systolic blood pressure, TD=treatment difference

¹ANCOVA model

²Logistic regression model

³Definitions of apnoea and hypopnoea from the 2007 American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events were used:

Apnoea events = pauses in breathing lasting ≥10 s

Hypopnoea events = pauses/reduction in breathing lasting ≥10 s and associated with a 4% decrease in oxygen saturation

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The incretin hormone GLP-1 is upregulated in critically ill ICU patients and regulates the metabolic response during acute inflammation: central role of IL-6

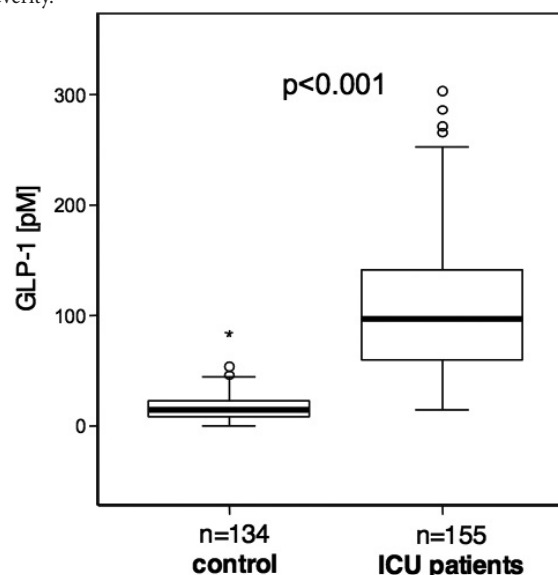
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Background and aims: Hypoglycemia predicts adverse outcome in critically ill patients including those with sepsis or myocardial infarction. The underlying mechanism for inflammation-mediated hypoglycaemia are incompletely understood. Experimental data suggest that hyperinsulinemia might play a role here and that LPS induces insulin secretion in a glucose-dependent manner. Since the incretin hormone Glucagon-like-peptide 1 (GLP-1) also causes insulin secretion, we speculated that GLP-1 may be of relevance for inflammation dependent hypoglycaemia.

Materials and methods: C57BL/6J, IL6 knockout (KO) and IL1 receptor KO mice were given i.p. injections of saline or lipopolysaccharides (LPS) (100µg/kg), IL1β, IL6 and TNFα (all 4 µg/kg), respectively (*n*=6–12/ group). A total of 155 critically ill patients (112 with sepsis, 43 without sepsis) were studied prospectively upon admission to the medical intensive care unit (ICU) and compared to 134 healthy controls.

Results: LPS upregulated GLP-1 secretion in C57BL/6J mice with a maximal 3,4 fold increase ($p<0.001$) 120 minutes post LPS injection, which was paralleled by elevated insulin ($p<0.001$) and decreased glucose ($p<0.001$) levels. A similar increase of serum GLP-1 and insulin was found after IL1β or IL6 administration. Studies in IL1-R-KO - and IL6 KO mice showed that LPS dependent GLP-1 secretion was selectively dependent on IL6 but not on IL1 secretion. Consistently IL6 ($p<0.05$) but not IL1β or LPS stimulated GLP-1 secretion from intestinal L cells in vitro. To evaluate the functional relevance of GLP-1 for glucose metabolism we inhibited its degradation by administration of the DPP4 inhibitor sitagliptin (40mg/kg), which augmented LPS-induced insulin secretion and blood glucose lowering in mice. Conversely, injection of the GLP-1 receptor antagonist exendin 9-39 (100nM/kg) markedly blunted LPS-dependent increase of serum insulin and prevented endotoxic hypoglycaemia. We next asked whether GLP-1 would also be upregulated in humans under inflammatory conditions. Indeed, critically ill patients admitted to the intensive care unit showed an 6.9 fold increase of total GLP-1 plasma levels in comparison to healthy controls (97.0 pM versus 13.9 pM; $p<0.001$). Among the ICU cohort higher GLP-1 plasma concentrations were found in patients presenting with sepsis (101.1 pM versus 67.5 pM in none septic patients) and more severe disease (109 pM in patients with Apache II > 10 versus 73.4 pM in patients with Apache II < 10; $p=0.002$). GLP-1 levels were associated with IL6 concentrations ($p<0.001$) in the whole ICU cohort and with insulin ($p<0.05$) in none septic ICU patients.

Conclusion: GLP-1 provides a new metabolic cross talk between the gut and the immune system with LPS dependent hypoglycaemia being dependent on an inflammatory cascade including IL6, GLP-1 and insulin. GLP-1 plasma concentrations are elevated in critically ill patients, correlating with disease severity.



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Saxagliptin in patients with prior heart failure - observations from the SAVOR-TIMI 53 trial

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Background and aims: Heart failure (HF) and T2DM frequently co-exist; however, there are limited data regarding the safety of an antihyperglycemic drugs in patients with T2DM and prior HF and thus clinical uncertainty over optimal glycemic control strategies.

Materials and methods: The SAVOR-TIMI 53 trial evaluated the safety and efficacy of saxagliptin vs. placebo in 16,492 patients with T2DM, at high risk of cardiovascular disease, of whom patients with HF at baseline were a pre-specified sub-group. The 1° endpoint was CV death, MI, or ischemic stroke. The 2° endpoint included the 1° endpoint together with hospitalization for HF, unstable angina, or coronary revascularization. Median follow-up was 2.1 years. The 1° and 2° endpoints were adjudicated by a blinded events committee.

Results: 2,105 patients (12.8%) reported prior HF at baseline. Patients with prior HF were older and more likely to have established CV disease and renal impairment, with no difference in duration of DM or HbA1c. More patients with prior HF were on ASA, statins, B-blockers, and insulin, but fewer on metformin or sulfonylurea. There was no difference in the duration of DM and only a small difference (0.1%) in baseline HbA1c. Patients with prior HF were at double the risk for all CV events compared to patients with no prior HF. (Table) The relative effect of saxagliptin versus placebo was similar in patients with and without prior HF for the 1° and 2° endpoints. The increased risk of hospitalization for heart failure with saxagliptin was also similar regardless of prior HF, though the absolute risk difference with saxagliptin was greater in patients with prior HF. The incidence of hypoglycaemia, adverse events, and A1c reductions are presented in the Table.

Conclusion: Despite being at substantially increased risk of CV complications, saxagliptin neither increased nor decreased the risk of the 1° and 2° endpoints compared to placebo in patients with T2DM and prior HF. Similarly, there was no relative differential in the increased risk of hospitalization for HF with saxagliptin, though the absolute risk was greater. These data provide important information regarding the safety and efficacy of an antihyperglycemic agent in a traditionally difficult to treat population.

	Prior Heart Failure (n=2105)				No Prior Heart Failure (n=14387)				Interaction p-value
	Saxa. (yr- KM%)	Plac. (yr- KM%)	HR	P-value	Saxa. (yr- KM%)	Plac. (yr- KM%)	HR	P-value	
1° Endpoint	13.9	12.3	1.13	0.32	6.4	6.5	0.97	0.61	0.28
2° Endpoint	23.9	22.9	1.06	0.50	11.2	10.9	1.01	0.87	0.63
CV Death	7.6	7.3	1.03	0.88	2.6	2.3	1.04	0.73	0.94
Hospitalization for Heart Failure	11.7	10.2	1.21	0.15	2.3	1.7	1.32	0.02	0.67
Minor/Major Hypoglycemia	15.7	14.0	1.20	0.12	15.0	13.3	1.16	0.001	0.83
Major Hypoglycemia	3.0	2.3	1.47	0.14	1.9	1.6	1.23	0.10	0.57
Hospitalization for Hypoglycemia	1.3	0.5	2.55	0.04	0.5	0.5	1.02	0.94	0.09
Adverse Events	Saxa. (n/N%)	Plac. (n/N%)	OR	P-value	Saxa. (n/N%)	Plac. (n/N%)	OR	P-value	Interaction p-value
Any Adverse Events	74.7	73.1	1.09	0.40	73.5	73.7	0.99	0.81	0.39
Serious Adverse Events	34.9	34.1	1.04	0.69	24.4	23.9	1.02	0.56	0.89
	Saxa. (%)	Plac. (%)		P-value	Saxa. (%)	Plac. (%)		P-value	
A1c Reduction at 2yrs (median)	-0.3%	-0.1%		<0.001	-0.3%	0%		<0.001	
A1c <7 at 2 yrs	38.6	27.4		<0.001	37.1	28.0		<0.001	

1° Endpoint - CV Death/ MI/ Ischemic Stroke

2° Endpoint - CV Death/ MI/ Ischemic Stroke/ Hospitalization for UA, Heart Failure, Revascularization

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OP 32 Complications: expect the unexpected

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A randomised clinical trial on the efficacy of an adapted bowel preparation for diabetic patients undergoing a colonoscopy

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Background and aims: Need for colonoscopy is common among diabetic patients because of the high prevalence of gastrointestinal symptoms but they are more likely to have inadequately cleansed bowels which leads to a repeat procedure that will increase patients' exposure to unnecessary risk and costs. No studies have evaluated the importance of dietary recommendations before the procedure or the need for hypoglycaemic drug adjustments during preparation. A specific bowel preparation for patients with diabetes with special attention to dietary recommendations and modification of hypoglycaemic medication could facilitate patient adherence and consequently improve bowel cleansing. We evaluated the efficacy and safety of an adapted protocol for colon cleansing in diabetic patients.

Materials and methods: Single-blind experimental design, 80 persons with diabetes who were scheduled for colonoscopies were randomly assigned to either the experimental diabetic colon preparation (DMe) or the standard colon preparation (DMs). A group of 40 non-diabetic subjects was also included (noDM). DMe patients were instructed to follow a specific low-residues diet, with special attention to carbohydrate intake, without liquid diet the day before colonoscopy, and to modify the doses of hypoglycaemic medication prior to the procedure. DMs subjects received the standard recommendations including general information on low-residues diet and liquid diet the day before the procedure with no counselling on hypoglycaemic medication adjustments. All patients received the same medication for bowel preparation. Endoscopists blinded to the type of preparation scored the type of residual stool and the percentage of bowel wall visualized for each segment of colon and for the overall examination using the Boston score. Hypoglycaemic episodes during preparation were recorded.

Results: 73 diabetic patients were randomized: 37 in DMe group and 36 in the DMs group. 40 noDM subjects were also included. NoDM patients were younger (56±15 vs 68±10 years, p<0.001), less frequently male (55% vs 74%, p<0.001) and had a lower comorbidity index (1 vs 14, p=0.07) than diabetic subjects. Subjects in the DMe and DMs groups were comparable in all the analyzed characteristics (table). Overall, the noDM group had a higher Boston score than diabetic subjects (8,3±1,5 vs 7,3±2, p=0.010). DMe patients had a higher Boston score than DMs (7,6±1,5 vs 6,4±2,8, p=0.02) a lower number of non-diagnostic colonoscopies (2,8% vs 19,4%, p=0.028) which was similar to that of noDM subjects (2,7%, p=0.93).

Conclusion: An adapted bowel preparation protocol for diabetic patients undergoing a colonoscopy can achieve a better visualization of the colon wall, which in turn reduces the need for repeat procedures to a level equivalent to the general population.

	Non-diabetic N=40	DM intervention N=36	DM standard N=37	P**
Age (years)	56.15*	68.10	68.10	0.232
Male, n (%)	22 (55)*	24 (67)	30 (80)	0.071
BMI kg/m ²	23.915	24.313,6	23.314,6	0.388
Charlson index >1, n (%)	1 (2.5)*	13 (36.1)	15 (40.5)	0.097
Constipation, n (%)	11 (27.5)	6 (16.6)	8 (21.6)	0.591
Elementary studies, n (%)	22 (55)	17 (47.2)	15 (40.5)	0.824
Diabetes characteristics				
Diabetes duration, years		12.1110	9.717	0.618
Insulin treatment, n (%)		8 (22.2)	6 (16.2)	0.798
Chronic complications, n (%)		9 (25)	7 (18.9)	0.841
Boston score	8.315*	7.615	6.412,8	0.020
Non diagnostic colonoscopy, n (%)	1 (2.5)	1 (2.7)	7 (19.4%)	0.028
Hypoglycaemic episodes, n		1	1	1

*p value <0.05 for comparisons between non-diabetic and diabetic subjects.

** for comparison between DM intervention group and DM standard preparation group.

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Dissociation pattern in resting-state default mode network connectivity in type 2 diabetes patients

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Background and aims: Type 2 diabetes mellitus (T2DM) has been shown to be associated with an increased risk of cognitive impairment. Patients with impaired cognition often show default-mode network (DMN) disruption. This study aimed to investigate the integrity of the DMN by using independent component analysis (ICA) methods in patients with T2DM and to correlate the DMN functional connectivity (FC) changes with neurocognitive performance and clinical variables.

Materials and methods: The current study was approved by the local ethics committee and written informed consent was obtained from all participants. Twenty-nine T2DM patients and thirty well-matched healthy controls were included in the study and underwent resting-state functional MRI (rs-fMRI) in a 3 Tesla unit. All participants underwent a detailed battery of neuropsychological tests. Clinical parameters such as plasma glucose, HbA1c, insulin resistance, BMI and cholesterol levels were also collected. A group ICA method was used to extract the DMN of all participants. Two components were identified to be related to two sub-networks of the DMN, including the anterior and posterior parts of the DMN. Z-maps of the two sub-networks were compared between the two groups and correlated with the neurocognitive performance and clinical parameters by using Pearson correlation analysis.

Results: Patients with T2DM showed significantly increased frontal connectivity around the medial prefrontal cortex (MPFC) in the anterior DMN (aDMN) and significantly decreased connectivity in the posterior cingulate cortex (PCC) and angular gyrus in the posterior DMN (pDMN) (Figure 1). The FC strength in aDMN was found to be negatively correlated with the score on complex-figure-test ($r=-0.46$) and positively correlated with the FPG ($r=0.44$). On the other hand, the FC strength in pDMN was negatively correlated with the disease duration ($r=-0.55$) and the time spent on trail-making-test (part B) ($r=-0.68$). These associations were independent of vascular risk factors and cerebral small vessel disease.

Conclusion: The current study demonstrated the dissociation between anterior and posterior DMN sub-networks in patients with T2DM. Our results highlight the important role of the DMN in the pathophysiology of T2DM-related cognitive impairment and suggest that abnormal DMN activity may be a trait associated with T2DM patients.

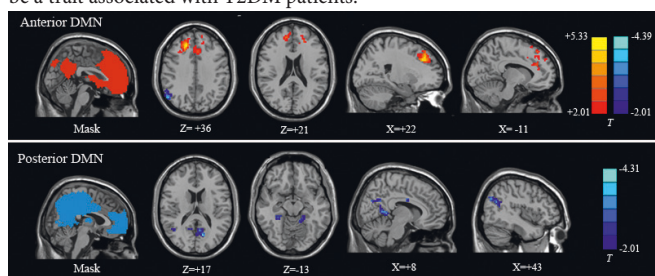


Fig.1 aDMN (upper row) and pDMN (lower row) differences between T2DM patients and healthy controls ($P < 0.05$, AlphaSim corrected)

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Disrupted circadian arousal patterns comorbid with diabetesM. Kadono¹, G. Hasegawa², M. Fukui³, N. Nakamura³;

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Background and aims: Sleep-wake disturbances and disruptions of circadian rhythms are now recognized to be highly prevalent comorbidities in patients with medical illness. Likewise, it is supposed that circadian arousal rhythms were disrupted in parallel with metabolic and vascular vulnerability in diabetes. We aimed to explore disrupted circadian arousal patterns comorbid with diabetes.

Materials and methods: Ninety-one diabetic outpatients with BMI < 25 kg/m² in our diabetes clinic wore an actigraph for consecutive 7 days (men: 41, type 1 diabetes: 3, age: 68.7 ± 8.2 yrs). Patients with depression, dementia, liver cirrhosis, renal failure, blindness, and shift work were excluded. Activity recording derived from an actigraph was aggregated in hourly bins counting

the number of minutes containing any activity. Next, an average 24-hour arousal profile was calculated by averaging each corresponding hour of all recorded days. To determine several underlying circadian arousal patterns by the 24-hour arousal profiles, a principal component analysis was applied. The analysis identified three factors which explained 25.2%, 17.7 %, and 19.8% of the total variance. These components were interpreted as 1) a “Daytime Arousal” factor (DA) with positive loadings of hourly activity counts of 8:00 a.m.-19:00, 2) a “Midnight Arousal” factor (MA), with positive loadings of 0:00 a.m.-4:00 a.m., and 3) a “Phase” factor (P), with positive loadings of 18:00 - 0:00 a.m., and inverse loadings of 4:00 a.m.-7:00 a.m.. In addition, a “Hyper-arousal” (HA) factor, average total time spent awake per day, was calculated from total activity counts across a day. Last, the stepwise regression analysis was used to evaluate associations of diabetic clinical backgrounds with the circadian rhythms related measurements, DA, MA, HA and P.

Results: Higher age, higher BMI, higher cholesterol levels, insulin treatment were associated with lower levels of DA ($p=0.04, 0.03, 0.01$ and 0.02 , respectively), while higher HbA1c and the presence of painful neuropathy was associated with higher DA ($p=0.03$ and 0.02). Male gender and higher levels of urinary albumin excretion were associated with higher MA ($p=0.04$ and 0.03). Similarly to DA, lower age, lower BMI, higher HbA1c, no insulin treatment, and painful neuropathy were associated with higher HA ($p=0.05, 0.02, 0.06, 0.01$, and 0.02). Furthermore, painful neuropathy and antilipids usage were independent predictors of delayed P ($p=0.02$ and 0.03), while cardiovascular diseases and smoke were independent predictors of advanced P ($p=0.04$ and 0.01).

Conclusion: Diabetic clinical backgrounds were associated with a variety of circadian arousal patterns and daily arousal levels. The current study suggests that high age, obesity, dyslipidemia, and insulin treatment in diabetes might reduce daily arousal levels with excessive daytime sleepiness, and that poorly controlled blood sugar and painful neuropathy in diabetes might be pathogenically associated with sleep loss resulting from short sleep or insomnia which is a 24-hour disorder of hyperarousal. Furthermore, diabetic neuro-vasculopathies may alter circadian arousal patterns by affecting the midnight arousal levels and circadian phase. These findings could provide a novel insight helpful for interventions for various patterns of sleep/wake disorders comorbid with diabetes.

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Sleep stages and type 2 diabetes: characterisation of electroencephalography featuresA. Lecube¹, O. Mestres², A. Ciudin³, C. Zafon⁴, G. Sampol², O. Romero², F. Rius⁵, C. Hernández², A. Casteràs⁴, A. Caixàs⁶, R. Simó³;

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Background and aims: There is growing evidence suggesting that type 2 diabetes mellitus (T2DM) is an independent risk factor for severe nocturnal hypoxemia and sleep breathing disorders. However, it is unknown whether or not these sleep disturbances affect the structure of sleep.

Materials and methods: For this purpose we designed a case-control study between 28 patients with T2DM and 56 non-diabetic subjects, closely matched by age, gender, BMI, waist and neck circumferences, and smoking status. Subjects were also matched for their apnea-hypopnea index (IAH) [31.2 (1.0 to 106.3) vs 33.9 (1.2 to 109.1) events per hour, $p=0.924$]. The exclusion criteria included chronic respiratory disease, neuromuscular and cerebrovascular disease, alcohol abuse, use of sedatives, and pregnancy. Examination included an electroencephalography and respiratory polysomnography, as well as oxygen saturation measures and the degree of sleepiness using the Epworth Sleepiness Scale (ESS). Studies with less than 5 hours of correct signal recording were ruled out.

Results: No differences in the Rapid Eye Movement (REM) Sleep (13.5 ± 8.2 vs. 12.8 ± 7.4 % of total time of sleep, $p=0.689$) nor in the Non-Rapid Eye Movement (NREM) Sleep stages (stage 1: 18.6 ± 12.4 vs. 15.1 ± 13.0 %TTS, $p=0.243$; stage 2: 58.1 ± 14.0 vs. 59.3 ± 12.5 %TTS, $p=0.688$; stage 3: 12.5 ± 10.5 vs. 12.3 ± 10.2 %TTS, $p=0.924$; stage 4: 13.8 ± 10.8 vs. 12.4 ± 10.4 %TTS, $p=0.594$) were observed between subjects with and without T2DM. However, T2DM patients showed a higher number of microarousals (or brief awakenings) events than control subjects [34.1 (4.6 to 101.4) vs. 22.7 (1.3 to 93.1)

events per hour during the total time of sleep, $p=0.023$]. A significant positive correlation between microarousals and the AHI was detected ($r=0.807$, $p<0.001$). Finally, a stepwise regression analysis showed that both the AHI and the presence (or not) of T2DM (but not gender, age, BMI, neither neck circumference) independently predicted microarousals ($R^2=0.593$).

Conclusion: T2DM adversely affects structure of sleep, becoming an independent risk factor for higher rates of microarousals. As sleep fragmentation has been involved with increased levels of lipids and blood pressure, this increased rate of microarousals may be implicated in the progression of cardiovascular disease in T2DM.

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Diabetes is associated with increased prevalence of perinasal sinus diseases

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Background and aims: Past studies have reported that patients with diabetes are susceptible to respiratory tract infections. However, it remains unknown whether or not individuals with diabetes are more likely to have perinasal sinus diseases than those without. In the present study, we investigated the association between diabetes and perinasal sinus diseases in Japanese adults who underwent a health checkup.

Materials and methods: This was a cross-sectional study performed in the medical checkup unit of one general hospital in Japan. A total of 1,350 adults who underwent a medical checkup including a head MRI scan from January 2007 to December 2011 were enrolled in the study. The participants were generally healthy and asymptomatic. Information on lifestyle was obtained by a questionnaire. Anthropometric data and blood samples were collected under a fasting condition. Diabetes was defined by any of the following criteria: 1) self-reported diabetes or use of anti-diabetic medications based on the questionnaire, 2) fasting plasma glucose of ≥ 126 mg/dl, or 3) HbA1c of ≥ 6.5 %. Perinasal sinus diseases were defined as partial or complete opacification of perinasal sinuses (including mucosal thickening and small polyps) detected by a head MRI scan. The prevalence of perinasal sinus diseases in adults with diabetes and those without was calculated, which was standardized to the 1985 model population of Japan. Multiple logistic regression analysis was performed to calculate the odds ratio of having perinasal sinus diseases in adults with diabetes in relation to those without. The model was adjusted for age, sex, body mass index, waist to hip ratio, smoking status, alcohol intake and white blood cell count. In addition, the dose-response relationship was examined between HbA1c levels and the presence of perinasal sinus diseases.

Results: Of the 1,350 adults (mean age, 62 ± 10 years; 72% men), 220 diabetes cases were identified. Perinasal sinus diseases were diagnosed in 151 adults. The age-standardized prevalence of perinasal sinus diseases was 18.8% in adults with diabetes and 11.0% in those without, respectively. In the logistic regression analysis, a significant association was observed between diabetes and the presence of perinasal sinus diseases after adjustment for the confounders [odds ratio (OR) = 1.74, 95% CI = 1.12–2.71]. The odds of having perinasal sinus diseases increased with HbA1c levels. Compared with adults with HbA1c of <5.5 %, those with HbA1c of 5.5–6.5% and ≥ 6.5 % were more likely to have perinasal sinus diseases with ORs of 1.32 (95% CI=0.88–1.97) and 1.86 (95% CI=1.05–3.31), respectively (p for trend = 0.03).

Conclusion: The present study found that perinasal sinus diseases are more common in adults with diabetes than those without. The dose-response relationship between hyperglycaemia and the presence of perinasal sinus diseases was also observed. The association was independent of known risk factors for perinasal sinus diseases. Although the clinical significance of the diseases detected by a head MRI scan remains undetermined, physicians should keep in mind the higher prevalence of perinasal sinus diseases among diabetic patients in daily practice.

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Hyponatraemia is associated with all-cause mortality in patients with type 2 diabetes (ZODIAC-46)

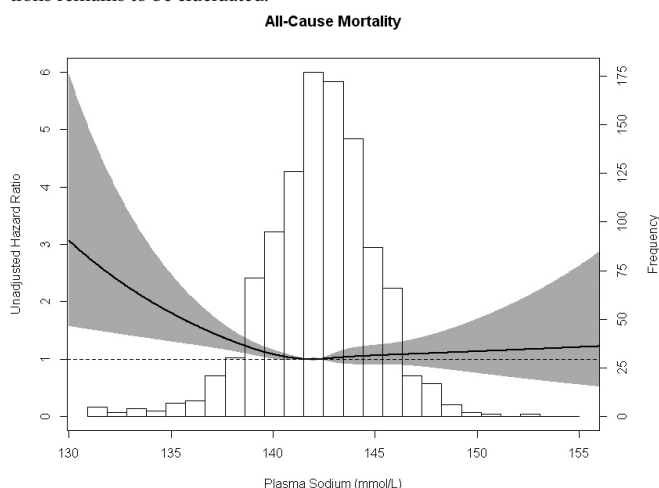
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Background and aims: Hyponatremia (i.e. plasma sodium <135 mmol/L), the most prevalent electrolyte disorder in clinical practice, was found to be an independent predictor for all-cause mortality both in patients with heart failure and in the general population. Although hyponatremia appears more prevalent in diabetes, the association of hyponatremia with mortality in patients with type 2 diabetes is rarely studied. Our aim was to prospectively investigate whether plasma sodium is associated with cardiovascular (CV) and all-cause mortality in patients with type 2 diabetes.

Materials and methods: Patients with type 2 diabetes participating in the Zwolle Outpatient Project Integrating Available Care (ZODIAC) study were included. Cox regression analyses with restricted cubic splines were used to investigate the association of baseline plasma sodium with CV and all-cause mortality.

Results: We included 1,068 patients (45% male, age 67 ± 12 years). Mean plasma sodium concentration was 142.3 ± 3.0 mmol/L. In multivariable linear regression analyses, plasma sodium was associated with coeppin, a surrogate marker for vasopressin, urinary albumin-to-creatinine ratio (ACR), HbA1c and gender. After median follow-up for 6.5 [interquartile range: 3.1–10.2] years, 345 patients died (32%), with 145 deaths (14%) attributable to CV causes. A restricted cubic spline depicting the association of plasma sodium with all-cause mortality is shown in Figure 1. In univariable Cox regression analyses, plasma sodium levels <142 mmol/L were significantly associated with all-cause mortality (Hazard Ratio [HR] 0.90; $p=0.003$), but not with CV mortality (HR 0.90; $p=0.07$). After adjustment for age, sex, BMI, smoking, systolic blood pressure, total cholesterol-to-HDL ratio, duration of diabetes, HbA1c, use of antihypertensive medication including diuretics and RAAS inhibitors, history of CV diseases, serum creatinine, log ACR and coeppin, the associations of plasma sodium levels <142 mmol/L with all-cause mortality (HR 0.91; $p=0.04$) and CV mortality (HR 0.89; $p=0.09$) were essentially unchanged.

Conclusion: We found low plasma sodium levels to be independently associated with an increased risk for all-cause mortality in patients with type 2 diabetes. Whether hyponatremia itself leads to poor outcome or that it is a marker for (unidentified) co-morbidity severity or use of specific medications remains to be elucidated.



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OP 33 Device utilisation and outcomes

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Continuous intraperitoneal insulin infusion versus subcutaneous insulin for type 1 diabetes: a prospective, case-control trial proving non-inferiority

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Background and aims: Continuous intraperitoneal insulin infusion (CIPII) using an implantable pump is a last-resort treatment option for patients with type 1 diabetes mellitus (T1DM) who fail to reach acceptable glycaemic control with intensified subcutaneous (SC) insulin regimens. Aim of this study was to compare the effects of long-term CIPII with SC insulin therapy in T1DM.

Materials and methods: This is a 36 week, investigator initiated, prospective, open-label matched-control study. Patients were eligible if they had been treated with either CIPII or SC insulin for >4 years and had a HbA1c of ≥ 53 mmol/mol. CIPII treated patients were matched to SC treated patients regarding age and gender. In order to account for inequality between treatment groups, as CIPII treated patients are a selected group of patients in need of a last-resort treatment, the primary endpoint was not a superiority but a non-inferiority assessment of the difference in HbA1c between both groups. A non-inferiority margin of -5.5 mmol/mol was predefined. Secondary outcomes were clinical and biochemical parameters, quality of life (SF-36 (scores 0-100)) and treatment satisfaction (DTSQ (scores 0-36)). Analysis were performed with ANCOVA, taking baseline differences into account.

Results: During study, one patient withdrew consent. Subsequently 183 patients, 36% male with a mean age of 50 years (SD 1) and diabetes duration of 26 years (SD 13), of which 39 were treated with CIPII and 144 with SC insulin therapy were analyzed. Age and gender were well matched. Results are presented in table 1. HbA1c remained stable within the CIPII group while it decreased with -1.0 mmol/mol (95%CI -1.9, -0.1) in the SC group. Using ANCOVA, the difference between treatment groups was -3.0 mmol/mol (95%CI -5.0, -1.0) and met the predefined non-inferiority criterion. Besides a difference in alanine aminotransferase (ALT) concentrations between groups of 3.6 U/L (95%CI 1.2, 6.0) being higher in the CIPII group, no other differences were found. At baseline and at the end of the trial, SF-36 scores were lower among CIPII treated patients as compared patients treated with SC insulin. CIPII treated patients had lower SF-36 mental and physical component scores and higher treatment satisfaction as compared to patients treated with SC insulin at baseline and follow-up.

Conclusion: CIPII therapy is non-inferior to SC insulin therapy with respect to glycaemic control in the treatment of poorly controlled T1DM patients. Besides a lower ALT among SC treated patients, there are no differences in clinical and biochemical parameters. Despite a lower quality of life, treatment satisfaction is higher among CIPII treated patients. This study underlines the effectiveness of long-term CIPII therapy as last resort treatment in T1DM.

	CIPII		SC		MEAN DIFFERENCE BETWEEN GROUPS AT THE END (95%CI)
	BASELINE	END	BASELINE	END	
HbA1c (mmol/mol)	66.9 (14.4)	68.4 (14.1)	62.8 (8.9)	61.8 (9.4)	-3.0 (-5.0, -1.0)
SF-36 Mental component score	65.3 (18.0)	64.2 (17.1)	73.9 (16.4)	75.1 (14.4)	10.9 (5.2, 16.6)
SF-36 Physical component score	58.5 (18.8)	56.2 (21.5)	74.6 (16.8)	76.3 (16.2)	9.6 (4.1, 15.0)
Treatment satisfaction score	31.3 (3.4)	31.4 (3.6)	28.9 (5.3)	29.0 (4.8)	-2.8 (-4.5, 1.2)

Data is presented as mean (SD) and mean difference (95%CI)

Clinical Trial Registration Number: NCT01621308

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Three to four weeks of overnight closed loop insulin delivery during free living: analysis of randomised crossover studies in adults and adolescents with type 1 diabetes

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Background and aims: Results from single-night laboratory-based closed loop studies demonstrated improved overnight glucose control and reduced risk of hypoglycaemia in subjects with type 1 diabetes. Assessments outside hospital settings over longer periods in free living conditions are underway. We combined data collected during free-living unsupervised randomised open-label crossover studies comparing overnight closed loop and sensor augmented pump therapy.

Materials and methods: 40 participants with type 1 diabetes [24 adults recruited at three centres and 16 adolescents recruited at one centre (age 43 ± 12 years, HbA1c $8.0 \pm 0.9\%$; mean \pm SD) (15.6 ± 3.6 years, HbA1c $8.1 \pm 0.8\%$)] underwent training on study devices followed by two periods of sensor augmented pump therapy in combination with or without overnight closed-loop utilising a model predictive control algorithm to direct insulin delivery. The order of interventions was random; each period lasted four weeks in adults and three weeks in adolescents. Primary outcome was time when sensor glucose was in the target range between 3.9 and 8.0 mmol/L. All analyses were by intention to treat.

Results: Closed loop was started by participants on their own volition on 866 nights (89%). The proportion of time when sensor glucose was in target range (3.9–8.0 mmol/L) between midnight and 08:00 was increased by 18.5% during closed-loop compared to sensor augmented therapy ($P < 0.001$; Table). Closed loop significantly reduced mean overnight glucose by 0.8 mmol/L ($P < 0.001$), with no difference in glycaemic variability as measured by the standard deviation of sensor glucose. Time spent above target range was reduced ($P = 0.001$) and so was time spent in hypoglycaemia below 3.9 mmol/L ($P = 0.014$) during closed loop. Lower mean overnight glucose during closed loop was brought about by increased overnight insulin delivery ($P < 0.001$) without changing the total daily delivery ($P = 0.84$).

Conclusion: Overnight closed loop at home in adults and adolescents with type 1 diabetes is feasible, demonstrating improvements in glucose control and reducing the risk of nocturnal hypoglycaemia.

Table.

	Overnight closed loop (n=40)	Sensor augmented pump therapy (n=40)	P
Mean overnight glucose (mmol/l)	7.9 \pm 0.9	8.7 \pm 1.4	<0.001
SD of overnight glucose (mmol/l)	2.0 \pm 0.3	1.9 \pm 0.3	0.47
Time spent overnight at glucose levels (%)			
3.9 to 8.0 mmol/l	59.2 \pm 11.5	40.7 \pm 13.4	<0.001
3.9 to 10.0 mmol/l	77.4 \pm 8.6	61.8 \pm 13.3	<0.001
>8.0 mmol/l	37.9 \pm 12.4	53.8 \pm 17.0	0.001
<3.9 mmol/l	1.9 (0.7, 3.5)	2.9 (1.0, 6.4)	0.014
Insulin delivery overnight (U)	7.0 (5.4, 9.3)	6.0 (4.7, 7.4)	<0.001
Total daily insulin delivery (U)	40.3 (32.9, 52.6)	39.4 (32.8, 55.8)	0.84

Data shown are mean \pm SD or median (interquartile range)

Clinical Trial Registration Number: NCT01440140 & NCT01221467

Supported by: DUK, JDRF

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Impact of real-time continuous glucose monitoring system usage on endothelial function in adolescents with type 1 diabetes

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Background and aims: Chronic hyperglycaemia and recently defined “glucose variability” in the course of diabetes are established risk factors for cardiovascular disease. Children with type 1 diabetes (T1DM) are the high-risk group of accelerated early atherosclerosis development. Real-time continuous glucose monitoring system (RT-CGMS) provides with new possibilities for the detection of excessive glycaemic fluctuations and enables the patient to react quickly to decrease these adverse phenomena. The aim of the study was to assess the usefulness of RT-CGMS as an educational tool to find and reduce glycemic variability in order to improve the parameters of endothelial function in adolescents with T1DM.

Materials and methods: Forty T1DM patients aged mean 14.6 years, diabetes duration: 7.4 years, mean HbA_{1c} before the study: 9.35%, 19 boys and 21 girls were recruited. The study was based on one month continuous glucose sensors use combined with education of the patients and caregivers. Several parameters of glycemic variability (mean glucose, SD for the mean glucose, AUC>140 mg/dl, AUC<70 mg/dl, minimal and maximal glucose level) were analysed during first and last sensor use, together with brachial artery flow mediated dilatation (FMD), as a parameter of endothelial function. HbA_{1c} was measured before the study and after 3 months follow-up. Depending on the initial value of HbA_{1c} the group was divided into well-controlled group (with HbA_{1c} <7.5%, mean 7.25±0.19%) and poorly controlled group (with HbA_{1c} >7.5%, mean 9.88±1.22%). Decrease of HbA_{1c} by at least 0.5% after 3-month follow-up was the criterion to divide the study group into improved vs. not improved. To determine the differences between the study groups for variables with normal distributions the t-Student test was applied. The t-Student test for paired variables was used to compare the variables within the respective groups at baseline and after one-month (glycaemic variability parameters and FMD). To assess correlations between study parameters Pearson correlation coefficient was used.

Results: In the whole group FMD improvement was found (10.9% to 16.6%, p<0.005), together with significant decrease in all studied glycaemic variability parameters. Significant HbA_{1c} improvement was observed in 68% patients (27 children): 9.02% before vs. 8.04%, after study, P=0.001. In these patients compared to the group without improvement (10.0±1.7% vs. 10.4±1.4% after the study) we found greater increase of FMD (12% to 19%, P<0.005 vs. 8.2% to 11.3%, P=0.08) and greater decrease of the mean glucose, SD for the mean glucose, AUC>140mg/dl and maximal glucose level. In group with initial HbA_{1c} <7.5% we found greater improvement of FMD than in group with HbA_{1c} >7.5% (16% to 28%, p<0.005 vs. 9.6 to 13.9%, P<0.005). Improvement of parameters of glycemic variability in group with HbA_{1c} <7.5% was not so evident.

Conclusion: RT-CGMS can be considered as a new tool that allows to improve endothelial function in type 1 adolescent diabetic patients by enabling the quick reaction to decrease glycemic variability parameters in short-time observation. Whether such approach might influence to reduce the risk of future cardiovascular disease remains to be elucidated.

Supported by: Medical University of Białystok

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Insulin pumps (CSII) and cardiovascular diseases and mortality in the Swedish national diabetes register

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Background and aims: Insulin pump treatment (CSII) is given for improved glycemic control and quality of life. However, studies on the effect of CSII on long-term risk for cardiovascular diseases (CVD) and mortality are lacking.

Materials and methods: Comparison between 2136 patients with type 1 diabetes with CSII and 13,028 patients with insulin injections, from the Swedish National Diabetes Register (NDR), followed for outcomes during mean 5.9 years with 14,523 person-years.

Results: Baseline characteristics are given in the Table. Adjusted hazard ratios (95% confidence intervals) for fatal/nonfatal coronary heart disease and fatal/nonfatal cardiovascular disease (heart disease or stroke) were 0.95 (0.90-0.99; p=0.02), and for all-cause mortality 0.95 (0.91-1.00; p=0.03), when adjusting for age, sex, diabetes duration, baseline HbA_{1c}, systolic blood pressure, smoker, BMI, total cholesterol, history of CVD or heart failure at Cox regression. Hazard ratio were similar in a sensitivity analysis adjusting by stratification with a propensity score including these covariates and also HDL cholesterol, cumulative albuminuria, creatinine, antihypertensive drugs, and lipid-lowering drugs. P values for covariates between the groups after adjusting with the propensity score are given in the Table. Another sensitivity analysis in a subgroup with 15,164 patients with no history of CVD or heart failure also showed similar hazard ratios.

Conclusion: This large prospective observational study showed risk reductions of around 5% for cardiovascular diseases and mortality with CSII compared to insulin injections in patients with type 1 diabetes followed for 6 years.

Table. Baseline characteristics.

	Insulin pump	Insulin injection	P value ^a	P value ^c
Numbers	2,136	13,028		
Age, years	39.5 ± 12.4	41.8 ± 14.2	<0.001	0.46
Diabetes duration, years	25.2 ± 3.7	25.4 ± 3.9	0.1	0.54
HbA _{1c} , %	7.9 ± 1.1	8.0 ± 1.2	0.001	0.81
HbA _{1c} , mmol/mol	62 ± 12	64 ± 13	0.001	0.81
Systolic BP, mmHg	129.2 ± 16.8	126.6 ± 15.7	<0.001	0.06
BMI, kg/m ²	25.2 ± 3.7	25.4 ± 3.9	0.05	0.18
Total cholesterol, mmol/l	4.69 ± 0.87	4.80 ± 0.93	<0.001	0.52
HDL cholesterol, mmol/l *	85 ± 50	87 ± 52	0.1	0.62
Creatinine, µmol/l *	0.99 ± 0.63	1.14 ± 0.79	<0.001	0.31
Male gender	44.8	56.0	<0.001	0.61
Smoker	11.4	13.0	0.04	0.15
Albuminuria*	21.0	24.2	0.002	0.19
Antihypertensive drugs *	32.6	35.3	0.02	0.31
Lipid-lowering drugs*	22.4	25.1	0.009	0.45
History of CVD	4.9	7.6	<0.001	0.051
History of heart failure	0.75	2.0	<0.001	0.22

* Numbers were somewhat less due to missing data. ^a Significance using t-test or χ^2 test. ^b Significance using GLM after adjustment by stratification with a propensity score.

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The long term effect of continuous subcutaneous insulin infusion on renal function in type 1 diabetic patients with microalbuminuria

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Background and aims: The purpose of the study was to investigate whether insulin pump therapy (CSII) was superior to multiple daily insulin injections (MDI) to prevent progression of albuminuria in patients with type 1 diabetes (T1DM).

Materials and methods: Case-control prospective study of 3 years duration. 26 T1DM patients on CSII with AER of at least 20 µg/min were matched for age (40.3 ± 9.3 vs 42.2 ± 11.1 yrs), diabetes duration (27 ± 8 vs 24 ± 10 yrs) BMI (24.4 ± 3 vs 24.7±4.2 kg/m²) and AER with 26 T1DM patients on MDI. Patients were in stable maximal RAAS blockade before entering the study. Before the study all patients received education in intensive diabetes treatment and self-care including carbohydrate counting. Patients return to the clinic every 6 months for measurement of AER, GFR (by ioexol e.v.),

urinary concentrations of 8-iso-PGF2 α , HbA1c, 24hr blood pressure, 3 days Continuous Glucose Monitoring sensor (CGM), self monitored blood glucose profiles.

Results: AER decreased slightly in MDI group from a median value of 65 $\mu\text{g}/\text{min}$ (31–100 IQR) to 55 (33–155) after 3 years of follow-up. AER decreased significantly ($p<0.01$) from 63 $\mu\text{g}/\text{min}$ (37–154 IQR) to 18.3 (11–57) in CSII group. In particular only 3 patients on MDI regressed to normoalbuminuria at the end of follow-up, whereas 14 patients on CSII regressed to normoalbuminuria ($p<0.001$). GFR decline was faster in MDI group (-7.9 ± 9.9 ml/min/yr) than in CSII group (-3.1 ± 3.3 ml/min/yr; $p<0.05$). Urinary concentrations of 8-iso-PGF2 α were similar in the 2 groups and stable during the study. HbA1c was similar in MDI and CSII groups at entry into the study ($8.5\pm 1.5\%$ vs $8.2\pm 1.0\%$ respectively). Despite the thigh follow-up, metabolic control did not improve in both groups during the study: HbA1c remained unchanged (HbA1c at the end of the study: $7.8\pm 1.25\%$ in MDI group vs $8.0\pm 1.3\%$ in CSII group). All measures of glycaemic variability (Mean amplitude of glycaemic excursions [MAGE], Mean of daily differences [MODDs], Continuous overlapping net glycaemic action [CONGA], Low blood glucose index [LBGI]) were similar in the 2 groups throughout the study. Insulin daily dose was significantly higher in MDI than in CSII group both at entry (0.67 ± 0.39 vs 0.50 ± 0.11 U/Kg/day) and at the end of the study (0.67 ± 0.20 vs 0.54 ± 0.12 U/Kg/day; $p<0.01$). 24-hour blood pressure was well controlled in both groups and stable throughout the study. Mean of standard deviation of individual 24-hour blood pressure values, as a measure of blood pressure variability, were similar in both groups and stable during the follow-up period.

Conclusion: CSII, despite a similar blood glucose control, prevented the progression of albuminuria and the loss of GFR when compared to MDI in T1DM patients. The greater insulin requirement in patients on MDI suggests a condition of greater insulin resistance, a factor likely to contribute to the renal disease progression.

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Effect of insulin pump treatment on albuminuria and kidney function in type 1 diabetes

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Background and aims: Treatment with continuous subcutaneous insulin infusion (CSII) may reduce urinary albumin excretion as compared to treatment with multiple daily injections (MDI). We aimed to investigate the effect of 3 years CSII treatment on albuminuria, HbA1c, and kidney function compared to MDI in a single centre clinical setting.

Materials and methods: Case-control study of all type 1 diabetes patients initiating CSII treatment from 2004–2010 at our outpatient clinic and followed for at least three years. We identified 193 patients and matched them (1:2) to 386 patients treated with MDI in the same period. Matching was based on diabetes duration, gender, HbA1c and normo-, micro- or macroalbuminuria at baseline. Urinary albumin creatinine ratio (UACR) was measured yearly and annual change assessed from linear regression. Unpaired t-test and adjusted ANCOVA compared treatment groups.

Results: At baseline, both treatment groups included 39% men with diabetes duration of (mean \pm SD) 23 ± 12 years and a frequency of normo-, micro and macroalbuminuria of 84%, 12% and 4% respectively. Patients were (CSII vs. MDI) 48 ± 12 vs. 44 ± 11 years old, HbA1c 68 ± 11 vs. 68 ± 10 mmol/mol, eGFR 100 ± 23 vs. 101 ± 25 mL/min/1.73m², UACR (median [IQR]) 9 [6–19] vs. 9 [6–17] mg/g, and numbers on RAAS-treatment were 40 vs. 38%, ($p>0.58$ for all; except age; $p<0.001$). Annual change in UACR in CSII vs. MDI treated patients was (mean (CI95%)) -11.3 (-14.6 ; -8.0)% vs. -1.1 (-3.3 ; 1.1)%, ($p<0.001$). This remained significant after adjustment for diabetes duration, age, gender and baseline values of eGFR, systolic blood pressure, HbA1c and RAAS-treatment ($p<0.001$). Adjustment for average follow up values instead of baseline values did not change the significance ($p<0.001$). The yearly change in eGFR was -1.9 ± 6.7 vs. -1.8 ± 4.4 mL/min/1.73 m² ($p=0.73$) and in HbA1c -1.2 ± 2.8 vs. 0.2 ± 2.3 mmol/mol ($p<0.001$). Number of patients starting RAAS-treatment during follow up was similar in CSII vs. MDI treated; 10.9 vs. 10.4 % ($p=0.85$). In multiple regression analysis, adjusted for baseline values, a larger decline in UACR was significantly associated to CSII treatment and higher UACR ($p<0.001$). After adjustment for follow up values, a larger decline in UACR was significantly associated to CSII treatment ($p<0.001$), lower average HbA1c ($p=0.02$) and stable RAAS-treatment ($p=0.04$). In adjusted analyses restricted to patients on stable RAAS-

treatment during follow up ($n=465$) only CSII treatment was significantly associated to a larger decline in UACR ($p<0.001$).

Conclusion: In a case control study treatment with CSII over 3 years independently reduced UACR and HbA1c compared to MDI. In these mostly normoalbuminuric patients, change in kidney function was small, and similar, in the groups. The reduced UACR was only partly explained by reduction in HbA1c and may in addition be due to less glycaemic variability. This cannot be assessed from these data. The effect of CSII treatment on albuminuria needs investigation in randomized controlled trials.

OP 34 Novel approaches for beta cell protection

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Specific overexpression of the calcium-sensor sorcin in pancreatic beta cells protects against ER-stress and diet-induced type 2 diabetes

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Background and aims: Pancreatic beta cells use electrical signals to couple changes in blood glucose concentration to insulin release via extracellular calcium (Ca^{2+}) influx. Sorcin (SRI) is a Ca^{2+} -binding protein whose overexpression in cardiomyocytes rescues the abnormal contractile function of the diabetic heart and plays a role in terminating Ca^{2+} -induced Ca^{2+} -release. We explored here the role of sorcin in regulating Ca^{2+} fluxes in pancreatic beta cells and its ability to protect against glucolipotoxicity *in vivo*.

Materials and methods: Two lines of transgenic mice were generated on a C56Bl/6 background permitting inducible overexpression of SRI cDNA with the TetOn-system specifically in beta cells. Animals bearing one (SRI-1) or ten (SRI-10) copies of the SRI transgene, and littermate controls (CTRL), were fed either a standard chow diet (SD) or a high fat diet (60% fat, HFD) and exposed to doxycycline in the drinking water (500mg/L) from 4 weeks onwards. Body weight measurements and fasting intraperitoneal glucose tolerance tests (1g/kg, IPGTT) were performed at regular intervals. Quantitative RT-PCR and real-time calcium imaging with Fura-Red (excitation light at 420 and 480nm, 40x/1.4NA objective and a cMOS camera on board an Olympus IX-71 microscope) were performed on islets isolated from either transgenic animals or from wild-type mice transduced with adenoviruses overexpressing GFP-SRI or GFP.

Results: Glucose tolerance during IPGTTs worsened considerably in CTRL males under HFD (120 min glycaemias (mmol/L): 12.3 ± 0.9 at 8-week-old, 22.1 ± 1.7 at 16-week-old, $n=17$) but not in CTRL males under SD (120 min glycaemia (mmol/L): 7.2 ± 0.2 at 8 weeks, 7.3 ± 0.5 at 16 weeks, $n=10$). Strikingly, and despite having similar body weights, male SRI-1 mice on HFD displayed improved glucose tolerance at 16 weeks of age (areas under the curve (AUC; a.u.): 111.9 ± 8.8 for SRI-1, 136.2 ± 7.3 for CTRL, $n=16$, $p<0.05$). SRI-10 males on HFD already displayed improved glucose tolerance after 8 weeks of age (AUC (a.u.): 73.0 ± 2.3 for SRI-10, 95.8 ± 5.3 for CTRL, $n=22$, $p<0.001$) and fasting glycaemia (0 min glycaemia (mmol/L): 8.7 ± 0.4 for SRI-10, 10.9 ± 0.8 for CTRL, $n=22$, $p<0.05$). mRNA levels of genes involved in endoplasmic reticulum (ER) stress, including GRP78 (fold-change normalised to beta-actin: 0.29 ± 0.13 , $n=11$, $p<0.05$) and CHOP (0.16 ± 0.03 , $n=11$, $p<0.001$), were reduced significantly in SRI-1 versus CTRL islets. Moreover, adenovirus-mediated SRI overexpression increased basal cytosolic Ca^{2+} levels in wild-type mouse islets ($F_{420/480}$ ratio (a.u.): 1.0 ± 0.02 vs 0.74 ± 0.01 , $n=23$ clusters/group, $p<0.001$ vs GFP virus-infected islets) at low (3 mM) glucose and increased the peak amplitude of Ca^{2+} rises in response to high glucose (20 mM; AUC (a.u.): 463.4 ± 14.9 vs 278.2 ± 6.0 , $n=23$ clusters/group, $p<0.001$) or depolarisation with KCl (20 mM; AUC (a.u.): 157.8 ± 5.0 vs 89.3 ± 2.3 , $n=23$ clusters/condition, $p<0.001$).

Conclusion: Our *in vivo* experiments reveal that targeted SRI overexpression preserves pancreatic beta cell function and whole body glucose homeostasis during high fat feeding. These actions involve the alleviation of ER stress and increases in intracellular Ca^{2+} . Such alterations may avert apoptosis and loss of beta cell mass during type 2 diabetes.

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Chaperones ameliorate beta cell dysfunction and amyloid formation associated with human islet amyloid polypeptide overexpression

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Background and aims: In type 2 diabetes and islet transplantation beta-cell dysfunction is thought to be due to several causes, one being the formation of toxic protein aggregates called islet amyloid, formed by accumulations of misfolded human islet amyloid polypeptide (hIAPP). The process of hI-

APP misfolding and aggregation is one of the factors that may perturb the endoplasmic reticulum (ER) homeostasis, leading to beta cell dysfunction and ultimately, cell death. Molecular chaperones have been described to be important for regulation of ER response to ER stress by stabilizing protein conformation and improving ER capacity. The aim of the present work is to determine whether chaperone treatment is able to counteract hIAPP-induced beta cell dysfunction and, ultimately, diminish amyloid formation and cell death in pancreatic islets.

Materials and methods: hIAPP Tg islets and INS1E cells, stably expressing hIAPP (hIAPP-INS1E) were cultured with 16mM or 25 mM glucose and 400μM palmitic acid and treated with chemical chaperones taurine conjugated ursodeoxycholic (TUDCA) and phenyl butyric acid (PBA) or endogenous chaperones BiP/GRP78 (BiP) or protein disulfide isomerase (PDI). Islet function was determined by glucose-stimulated insulin secretion and gene expression and protein levels of ER stress markers (CHOP, ATF3 and spliced XBP1) were analysed by real-time RT-PCR and Western blot. Wild type and hIAPP Tg islets were cultured for 7 days at 16mM glucose and treated with TUDCA or PBA. Amyloid formation was determined by ThioS staining.

Results: hIAPP-INS1E cells exposed to high glucose and palmitic acid showed an increase in ER stress when compared to INS1E cells expressing rat IAPP or INS1E control cells. Treatment with chaperones BiP, PDI, TUDCA or PBA alleviated ER stress and increased insulin secretion in hIAPP-expressing cells. Treatment of wild-type and hIAPP Tg islets with BiP, PDI, TUDCA and PBA increased insulin output in basal conditions after a glucose-stimulated insulin secretion. When hIAPP Tg islets were exposed to high glucose and palmitic acid, chaperone treatment was able to revert beta cell dysfunction by restoring insulin secretion. Moreover, when hIAPP Tg islets were cultured for 7 days at 16mM glucose, amyloid plaques were formed throughout the islet engaging $18.2 \pm 2.3\%$ of the insulin positive area. TUDCA and PBA treatment was able to diminish amyloid formation of hIAPP Tg islets to $5.3 \pm 0.9\%$ and $1.2 \pm 0.4\%$ respectively, indicating that chaperones may play an important role in preventing beta-cell dysfunction and amyloid formation associated to T2D.

Conclusion: Chaperones ameliorate induced ER stress, increase insulin secretion and ultimately, diminish amyloid formation in a context of hIAPP overexpression. These innovative approaches could reveal new therapeutic targets and aid in the development and evaluation of strategies to diminish ER stress and limit the damaging amyloid observed in islets before transplant or in type 2 diabetic patients.

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Acidic, photo-activated nanoparticles restore autophagy in beta cells exposed to high nutrient environment

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Background and aims: We have previously shown that chronic (>16 hour) exposure to fatty acids (palmitate and oleate) inhibit autophagic flux in the pancreatic beta-cell, leading to accumulation of autophagosomes and decreased autophagy-dependent turnover, resulting in beta-cell dysfunction. Further investigations determined that fatty acids induced lysosomal dysfunction, characterized by loss of lysosomal acidity, lysosomal swelling, and decreased activity of lysosomal pH-dependent proteases. In this study we tested the hypothesis that fatty acid-induced impairment of lysosomal acidification plays a central role in the arrest of autophagic turnover and impaired beta-cell glucose-stimulated insulin secretion. To address this hypothesis we developed lysosome-targeted nanoparticles (NPs) that release acid upon photo-activation.

Materials and methods: Acidic NPs were developed that expand in size after UV-light activation, exposing carboxylic acid residues in the polymer that mediate an acidifying effect. Rhodamine-labelled NPs were tested for uptake and localization into INS1 cells (beta-cell insulinoma line) using flow cytometry and confocal imaging with LysoTracker dye. Efficacy of UV-activation and NP toxicity in INS1 cells were tested using propidium iodide staining and flow cytometry. Lysosensor Yellow/Blue dye and ratiometric imaging/analysis was performed to determine if UV-activated NPs could affect lysosome acidity and size in INS1 cells exposed to 0.4mM palmitate for 18 hours. Also, p62 and LC3-II levels in INS1 cells were assessed by Western blot to test NP-induced improvement of autophagic flux. Lastly, human islets were exposed to 2:1 oleate:palmitate for 4 days, and glucose-stimulated insulin secretion was analyzed to determine if UV-activated NPs could improve beta-cell function after fatty acid exposure.

Results: The NPs were equilibrated in a dose-dependent manner in INS1 and beta-cells within 1–2 hours and localized to lysosomes as assessed by co-localization with LysoTracker staining. High concentration (250 µg/mL) of NPs induced significant cell death when UV-activated but was well-tolerated when not UV-activated, indicating successful activation of NPs in intact cells and allowing determination of a non-toxic treatment dose of 25 µg/mL NPs in cells. UV-activation of NPs in INS1 cells exposed for 18 hours to palmitate showed a significant rescue of lysosomal acidity and size compared to palmitate treatment alone or palmitate co-treated with NPs that were never UV-activated. Furthermore, palmitate-induced p62 and LC3-II accumulation were decreased following UV-activation of NPs, indicating downstream improvement of autophagic flux. Finally, UV-activated NPs were able to partially restore fatty acid-induced decrease in glucose-stimulated insulin secretion in human islets, suggesting that rescuing lysosomal acidity and autophagic flux with acidic NPs can improve beta-cell function.

Conclusion: The capacity of acidic NPs to restore lysosomal acidity, morphology and clearance supports an upstream role for impaired lysosomal dysfunction in the development of deregulated insulin secretion. Additionally, acidic NPs may have therapeutic value in diseases where a cellular defect in lysosomal acidity inhibits autophagic flux, such as the fatty acid-induced arrest of autophagic flux in the pancreatic beta-cell.

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Reversible changes in pancreatic islet structure and function produced by elevated blood glucose

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Background and aims: Hyperglycaemia is common to all forms of diabetes and results from impaired insulin secretion and aberrant glucagon secretion due to changes in pancreatic islet cell function and/or mass. The extent to which hyperglycaemia *per se* underlies these alterations remains poorly understood as previous studies have been restricted to in vitro culture systems, or animal models where diabetes is artificially induced by β-cell ablation and other confounding factors (e.g. obesity and insulin resistance) are common. We generated a mouse model in which diabetes could be rapidly and reversibly switched on and off to study the effect of hyperglycaemia on pancreatic islet structure and function.

Materials and methods: An inducible human activating K_{ATP} channel mutation was expressed in β-cells of adult mice. This inhibited insulin secretion and resulted in rapid onset of diabetes. Islet morphology, ultrastructure and electrophysiological characteristics were studied.

Results: 4-week exposure to hyperglycaemia was associated with a significant reduction in insulin-positive cells (% of islet area composed of insulin; control: 86.5±0.3% vs. diabetic 27.8±1.7%). This reflected a significant reduction in insulin mRNA and insulin protein levels in islets (P<0.05). Following exposure to chronic hyperglycaemia, islets became predominantly composed of glucagon-positive cells (control: 16.6±0.9% vs. diabetic 73.5±2.1%) consistent with increased glucagon mRNA and protein levels. These changes in insulin and glucagon expression did not reflect alterations in cell turnover (as assessed by Ki67 and apoptosis measurements). Instead, β-cell ultrastructure showed a striking reduction in insulin granule content. There was also a significant increase in dual insulin/glucagon-positive cells from 0.3±0.1% in control to 5.7±1.2% in diabetic islets (P<0.05). Lineage tracing showed these cells derived from β-cells and expressed β-cell transcription factors. Furthermore, they had functional β-cell, not α-cell, voltage-gated Na⁺ channels. Following 1 day or 4-weeks of diabetes, normoglycaemia was immediately restored (within 24h) by subcutaneous insulin or sulphonylurea therapy. Restoration of normoglycaemia with these anti-diabetic drugs prevented and fully reversed the changes in islet structure and function.

Conclusion: Chronic hyperglycaemia resulted in the partial loss of β-cell identity in a mouse model of β-cell dysfunction. Hyperglycaemia, rather than K_{ATP} channel activation, underlies these changes as they were all prevented, and even reversed by anti-diabetic drugs. This highlights the remarkable plasticity of β-cells, and their ability to alter their gene expression, structure and

function in response to changes in circulating glucose levels. These data suggest that rapid implementation of good glucose homeostasis in diabetes can preserve β-cell structure and function, and even reverse established changes.

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Dual roles of a DPP-4 inhibitor on cytoprotection and proliferation of pancreatic beta cells in a mouse model of beta cell injury/regeneration

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Background and aims: Dipeptidyl peptidase-4 inhibitors (DPP4i) increase pancreatic β-cell mass in mouse models of diabetes mellitus. However, it remains unknown which of cytoprotection or proliferation of β-cells contributes to the increase by DPP4i. In this study, we analyzed these two events individually using a mouse model that sequentially exhibits β-cell injury and regeneration.

Materials and methods: We generated transgenic mice expressing diphtheria toxin (DT) receptor (DTR) and a red fluorescent protein (tomato) specifically in β-cells (*Rip-Cre;Rosa26^{DTR/tTomato}*). To analyze cytoprotective effect of DPP-4i, the mice were pre-administered with a DPP-4i MK-0626 (3mg/kg/day, mixed in a chow) and then were treated with DT (20 µg/kg, i.p.) for β-cell ablation. Six days after β-cell ablation, pancreases were excised and subjected to morphological examination. To analyze pro-proliferative effect of MK-0626, we administered the mice with MK-0626 for 14 days starting on the day of DT injection. To assess the effect of MK-0626 on β-cells through pathways other than potentiating Glucagon-like peptide (GLP)-1 signaling, the same experiments were also performed also with a GLP-1 analog liraglutide (100 µg/kg, twice a day, s.c.). Molecular mechanism of β-cell protection was investigated using a β-cell line MIN6 cells expressing DTR.

Results: DT injection to *Rip-Cre;Rosa26^{DTR/tTomato}* induced death in 96% of tomato-positive cells and a decrease in β-cell mass to ~30% at day 6 and then recovery of β-cell mass to ~50% at day 14. In *Rip-Cre;Rosa26^{DTR/tTomato}*, blood glucose levels were not increased after β-cell ablation by DT. As regards to cytoprotective effect of MK-0626, MK-0626 administration significantly increased the number of surviving tomato-positive β-cell after DT ablation. Comparable cytoprotective effect was observed by liraglutide administration and there was no additive or synergistic effect by co-administration of MK-0626 and liraglutide. Mechanism of cytoprotection was evaluated by DT-induced cell death in MIN6 cells expressing DTR. Pretreatment of the cells with liraglutide significantly attenuated the decrease in cell viability by DT. Considering that caspase-3 activation was inhibited by liraglutide and that caspase-3 or -9 inhibitor attenuated DT-induced cell death, cytoprotective effect of GLP-1 is mediated by suppressing caspase-3 and -9 signaling. In addition, cytoprotective effect of liraglutide is significantly decreased by a PI3K inhibitor or a MEK inhibitor. As regards to β-cell proliferative effect of MK-0626, its administration significantly increased BrdU incorporation at 14 days after DT ablation.

Conclusion: MK-0626 increased β-cell mass by both inhibition of cell death and augmentation of proliferation in our mice. The cytoprotective effect of MK-0626 is suggested to be mediated by increased GLP-1 through PI3K and MAPK signaling.

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RNA-sequencing identifies Nova1 as a major splicing regulator in pancreatic beta cells

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Background and aims: Alternative splicing (AS) is a basic mechanism for the regulation of gene expression, affecting more than 90% of human genes. We have recently shown by exon array and RNA-sequencing that pancreatic beta cell exposure to the pro-inflammatory cytokines interleukin-1 beta (IL-1) +

interferon-gamma (IFN) modifies AS of >500 genes. Our recently published data indicate the presence of the Nova1 splicing factor in beta cells, but there is no information about its role on these cells. We have presently coupled specific knockdown (KD) of Nova1 with RNA sequencing to determine all splice variants and downstream pathways regulated by this protein in pancreatic beta cells.

Materials and methods: Nova1 expression was inhibited by 60% with the use of specific siRNAs. Three FACS-purified rat beta cell preparations (90–95% pure) were RNA-sequenced under control conditions or after a 48-hour KD of Nova1. Samples were sequenced on an Illumina HiSeq 2000 sequencer and the data analysed using Tophat mapper and Flux Capacitor. The Cufflinks software suite was used to identify potentially novel transcripts. Expression was considered changed if it fulfilled two criteria, namely $p < 0.05$ by the Benjamini-Hochberg-corrected Fisher testing and modified in the same direction in all samples. Beta cell function and viability was evaluated by glucose-induced insulin secretion, glucose oxidation and by the nuclear dyes Hoechst/PI.

Results: RNA-sequencing and the subsequent bioinformatics analysis identified 43,460 transcripts as expressed in primary beta cells, corresponding to 18,257 genes. Nova1 KD modified expression of 18% of these genes and altered the splicing of nearly 5000 transcripts. Pathway analysis indicated that these genes are involved in exocytosis, apoptosis, calcium signalling, splicing and transcription. Nova1 KD effects on splicing of key downstream genes such as Gabrg2 and Neurexin1 were confirmed by RT-PCR ($n=5$). In line with the observed pattern of gene expression, Nova1 silencing inhibited insulin secretion and voltage dependent Ca^{2+} current by respectively $40 \pm 5\%$ ($n=5$; $P < 0.05$) and $35 \pm 10\%$ ($n=28-33$; $P < 0.05$) but did not affect glucose oxidation suggesting a main effect of exocytosis. Importantly, Nova1 silencing induced apoptosis via the intrinsic or mitochondrial pathway in INS-1E cells, FACS-purified rat beta cells and human islet cells (increase in apoptosis of 6–20%; $n=4-6$; $P < 0.05$), basally and after cytokine treatment.

Conclusion: The present data indicate that Nova1 has a major role in beta cell function, controlling the splicing and expression of key genes involved in gene transcription, insulin release and apoptosis. These findings identify a novel layer of regulation of beta cell function, namely AS controlled by key splice regulators.

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OP 35 Hypertension in diabetes

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Diabetes and ambulatory blood pressure monitoring: a cross-sectional analysis of 68045 hypertensive patients in Spain

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Background and aims: Hypertension affects the majority of patients with diabetes and constitutes a major risk factor for vascular complications. Ambulatory blood pressure monitoring (ABPM) provides a high-quality approach in estimating the true levels of blood pressure (BP). Several population- and patient-based studies have showed the benefits of ABPM in exploring the relationship between BP and cardiovascular events. A series of reports dealing with diabetic patients have also shown a close correlation between ambulatory BP and diabetic complications. We aimed to assess such characteristics in comparison with non-diabetic hypertensives by using the Spanish Society of Hypertension ABPM Registry.

Materials and methods: We performed a cross-sectional analysis of a 68045 patient database from the Spanish Society of Hypertension ABPM Registry, a nation-wide network of 41200 primary-care physicians performing ABPM under standardized conditions in daily practice. We identified 12 600 (18.5%) hypertensive patients with diabetes.

Results: When compared with patients without diabetes, diabetic hypertensives exhibited higher systolic blood pressure (BP) levels in every ABPM period (daytime 135.4 vs. 131.8, and night-time 126.0 vs. 121.0 mmHg, $P < 0.001$ for both) despite they were receiving more antihypertensive drugs (mean number 1.71 vs. 1.23, $P < 0.001$). Consequently, diabetic patients suffered from lack of control of BP more frequently than non-diabetic subjects particularly during the night (65.5% vs. 57.4%, $P < 0.001$). Prevalence of a non-dipping BP profile (64.2% vs. 51.6%, $P < 0.001$) was higher in diabetic patients. In the other hand, prevalence of ‘white-coat’ hypertension in diabetic patients was 33.0%.

Conclusion: We conclude that there was a remarkably high prevalence of alterations in ABPM in patients with diabetes. Abnormalities in systolic BP, particularly during the night, and in circadian BP pattern could be linked with the excess of BP-related cardiovascular risk of diabetes. A wider use of ABPM in diabetic patients should be considered.

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Achievement of individualised blood pressure targets in patients with hypertension and comorbid type 2 diabetes in a real world setting: results of the DIALOGUE registry

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Background and aims: There has been a recent appreciation of individualized HbA1c treatment targets in patients with type-2 diabetes. Respective guidance for blood pressure Targets are inconsistent between different guidelines and decisions made should consider individual patient risk. To our knowledge there is neither clinical practice data on actually pursued blood pressure treatment targets nor on patient characteristics associated with either strategy.

Materials and methods: DIALOGUE is a prospective, observational, multicenter registry focusing on Treatment targets and their achievement in clinical practice. Physicians were asked at baseline on blood pressure treatment targets pursued and compared to actual blood pressure levels values achieved at 6 months.

Results: A total of 8,601 patients were considered for the analysis. For 3,343 of these (38.9%) a systolic blood pressure target of ≤ 130 mmHg (strict group), for 2,874 (33.4%) a target of > 130 to ≤ 135 mmHg (medium) and for 2,384 (27.7%) a target of > 135 to ≤ 140 mmHg (loose) was pursued. Patients in the strict target group were younger, had a shorter diabetes duration and less co-morbid disease (table). Overall 70.5% of those with a strict SBP target

also had a HbA1c target $\leq 6.5\%$ and 40.7% of those with a loose SBP target also had loose HbA1c targets. At the 6 months follow-up the mean (\pm SD) SBP was 133.4 ± 13.5 mmHg in the strict group (25% exceeding 140 mmHg), 135.7 ± 13.3 mmHg in the medium group (25% exceeding 140 mmHg) and 139.0 ± 15.0 in the loose group (25% exceeding 147 mmHg) ($p < 0.0001$).

Conclusion: The data illustrate that systolic blood pressure targets chosen in patients with type-2 diabetes consider patient characteristics and overall co-morbidity and are aligned with the corresponding HbA1c treatment targets. The lesser morbid patient are, the stricter the treatment targets.

Baseline	SBP ≤ 130 mmHg	>130 to ≤ 135 mmHg	>135 to ≤ 140 mmHg	n
Age (median, IQR)	64 (55–72)	66 (58–74)	68 (59–75)	<0.0001
Female sex (%)	46.1	44.9	45.5	0.60
HbA1c target $\leq 6.5\%$	70.5	21.2	16.6	<0.0001
HbA1c target >6.5 to $\leq 7.0\%$	21.8	65.9	42.7	<0.0001
HbA1c target >7.5 to $\leq 7.5\%$	7.7	12.8	40.7	<0.0001
Diabetes duration (months) (median, IQR)	61.4 (27.6–109.7)	71.2 (34.1–120.2)	72.6 (34.1–121.0)	<0.0001
CAD (%)	23.3	24.1	24.5	0.56
Stroke/TIA (%)	5.4	6.8	6.5	0.05
Heart failure (%)	11.6	12.8	16.6	<0.0001
PAD (%)	4.9	7.4	8.5	<0.0001

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Considering individual variability in multiple risk markers in short-term response to angiotensin-receptor-blockade improves renal risk prediction

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Background and aims: The current use of Angiotensin-Receptor-Blockers (ARBs) is targeted towards blood pressure in order to ultimately decrease renal and cardiovascular (CV) risk. However, ARBs have multiple (off-target) effects that may potentiate or counteract the ultimate ARB effect on renal and CV outcomes. The extent to which the response in these off-target markers varies within an individual is unknown. We therefore investigated variability in the response to ARBs in different risk markers, the congruency in response between the markers within an individual, and the relation to hard renal outcomes.

Materials and methods: We used data from the RENAAL trial and assessed the change in the primary target systolic blood pressure (SBP), and the off-targets, albuminuria, potassium, haemoglobin, cholesterol and uric acid after 6 months of treatment with losartan. Response was defined as a change in expected direction whilst non-response was defined as no effect or change in opposite direction. Renal outcome was defined as end-stage renal disease (ESRD) or doubling of serum creatinine and ascertained during a median follow-up of 3 years. The improvement in predictive performance of renal outcomes for each individual after adding ARB induced changes in on-target and off-target risk markers to a renal risk score, consisting of age, gender, baseline eGFR, albuminuria, SBP, HbA1c, and haemoglobin, was assessed by C-statistic and relative integrated discrimination improvement (RIDI).

Results: We included 531 (71%) patients (age 60.0 years, 38% female) who had complete measurements at baseline and month 6. A response was observed in SBP (61%), albuminuria (72%), potassium (66%), haemoglobin (72%), cholesterol (61%), and uric acid (47%). The proportion of individuals with off-target responses was similar among patients with SBP reduction compared to patients with SBP rise, suggesting that off-target responses were independent of SBP response. Moreover, correlations between SBP and off-target responses within an individual were weak (all $r < 0.2$). Adding the individual responses in on-target and off-target risk markers to a renal risk score significantly improved renal risk stratification (increase in c-statistic from 0.81 to 0.85, $p < 0.001$, and RIDI by 53.4%; $p < 0.001$). Similar results were observed in two independent trials with irbesartan, the IDNT and IRMA-2 trials.

Conclusion: The antihypertensive ARBs show multiple effects which vary between individuals and vary between parameters within an individual. Integration of these short-term ARB induced responses of each individual in

a multiple risk marker scores significantly improves renal risk prediction. These results highlight the importance of monitoring and targeting multiple renal risk markers in response to ARB therapy to optimize renal protection.

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Distinct systolic blood pressure trajectories in type 2 diabetes patients.

The Diabetes Care system cohort

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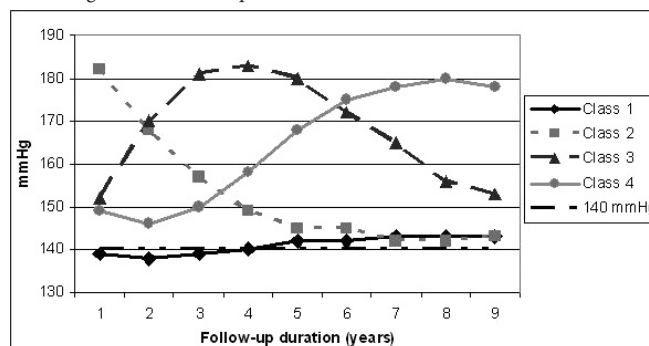
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Background and aims: Observational studies showed that only 40 to 60% of type 2 diabetes mellitus (T2DM) patients within primary diabetes care reach the recommended systolic blood pressure (SBP) target of ≤ 140 mmHg. In order to eventually improve blood pressure (BP) management, the aim of this study was to identify subgroups of T2DM patients with distinct trajectories of SBP levels. Subgroup characteristics were determined and the prevalence of microvascular complications and all-cause mortality rates over time in the different subgroups was investigated.

Materials and methods: 5711 T2DM patients with at least two SBP follow-up measurements were selected from a cohort of 9849 T2DM patients from the Diabetes Care system cohort in The Netherlands. The mean follow-up period was 5.7 years (range 2 - 9 years). Latent Class Growth Modelling was performed to identify subgroups of T2DM patients with distinct trajectories of SBP levels. Multinomial logistic regression analyses were conducted to determine subgroup characteristics. Associations of different subgroups with HbA1c level, retinopathy, microalbuminuria and medication use during follow-up were studied by constructing plots and by graphically comparing differences between classes.

Results: Four subgroups with distinct SBP trajectories were identified (Figure). The largest subgroup (85.6%) showed adequate SBP control (at or around 140 mmHg) over time. The second subgroup (5.6%) were hypertensive in first few years, responded slowly to BP management and eventually reached SBP control. The third subgroup (3.4%) showed deteriorating hypertension during the first four years, then showed insufficient response to BP management. The fourth subgroup (5.4%) showed deteriorating hypertension over time. Patients within subgroups 2-4, thus other than the adequate SBP control subgroup were significantly older, comprised more women, used more antihypertensive medication, had a higher prevalence of retinopathy and microalbuminuria and had higher all-cause mortality rates.

Conclusion: We identified four subgroups of T2DM patients with distinct SBP trajectories. More than 85% reached and maintained adequate SBP control. Subgroups with a more unfavourable course of SBP control also showed higher rates of retinopathy, microalbuminuria and all-cause mortality over time. This study identified important subgroups to target in order to improve BP management in T2DM patients.



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Relationships between the risk of cardiovascular events in type 2 diabetes and both visit-to-visit variability and time-to-effect in systolic blood pressure

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Background and aims: Recent studies, mostly in treated hypertensive patients, found a visit-to-visit BP variability is a risk factor for cardiovascular diseases (CVD) and organ damage independently of the mean BP. However, reports in patients with diabetes remain limited. Lack of a BP legacy effect on cardiovascular events has been reported in type 2 diabetes. However, data on the effects of early lowering of BP are still scarce. This study aimed to determine whether systolic BP (SBP) variability is able to predict, independently of the mean SBP, the incidence of CVD events in patients with type 2 diabetes, and to analyze the time-to-effect relationship between SBP and the risk of these events.

Materials and methods: A total of 652 (538 men, 114 women) patients with type 2 diabetes who first visited our hospital between 1995 and 1996, with at least 1 hospital visit per year and no history of CVD, and undergone 4 or more SBP determinations, were retrospectively enrolled. Patients were followed through June 2012. SD or CV was used as a measure of SBP variability. Risk of CVD events was evaluated by multivariate Cox proportional hazard models. SBP was analyzed as a mean value and a time-dependent covariate using the last observation carried forward or moving-mean during 1 year to 17 years preceding the events. For all analyses, SAS (version 9.3) was used.

Results: The mean values at baseline were age 55.7 years, duration of diabetes 5.6 years, BMI 23.4 kg/m², BP 133.7/77.9 mmHg, HbA1c 8.0%, Total-cholesterol (TC) 209.3 mg/dl, and HDL-cholesterol (HDL-C) 49.8 mg/dl. 275 patients were current smokers. The median follow-up period from first visit was 11.5 years, and the total number of SBP measurements was 53,949 (per-patient median: 78). By the end of follow-up, CVD events occurred in 71 patients. Hazard ratios for the incidence of CVD unadjusted, adjusted for age and sex, and for age, sex, duration of diabetes, current smoker, mean SBP, mean HbA1c, mean TC/HDL-C, mean BMI, and the number of SBP measurements (ln-transformed), increased across tertiles of SD and CV of SBP (Table). The incidence of CVD was significantly associated with SBP during the preceding 2 to 8 years, with the highest significance during the preceding 3 years, and borderline significance during the preceding 1 and the preceding 9 to over 17 years.

Conclusion: Visit-to-visit SBP variability was able to independently predict CVD events in patients with type 2 diabetes. Increased SBP during the preceding 3 years resulted in the highest risk of CVD; therefore, to prevent CVD, SBP management should focus on stable and well-timed control.

Table. Cumulative incidence and hazard ratios for cardiovascular diseases (CVD) associated with tertiles of SD and CV of systolic BP (SBP)

Outcome	Tertiles of SBP SD for incidence of CVD			p trend
	1 (n=217) <10.47 mmHg	2 (n=217) 10.47–13.19 mmHg	3 (n=218) >13.19 mmHg	
CVD	11 (5.1%)	21 (9.7%)	39 (17.9%)	<0.0001
		HR (95% CI)		
Unadjusted	1 (ref)	1.64 (0.79–3.41)	3.20 (1.64–6.26)	0.0002
Age- and sex-adjusted	1 (ref)	1.40 (0.67–2.92)	2.39 (1.19–4.81)	0.0074
Multivariate-adjusted ^a	1 (ref)	1.56 (0.72–3.38)	2.38 (1.14–5.00)	0.0145
Outcome	Tertiles of SBP CV for incidence of CVD			p trend
	1 (n=217) <8.145 %	2 (n=217) 8.145–9.892 %	3 (n=218) >9.892 %	
CVD	11 (5.1%)	22 (10.1%)	38 (17.4%)	<0.0001
		HR (95% CI)		
Unadjusted	1 (ref)	1.54 (0.74–3.19)	2.94 (1.50–5.75)	0.0006
Age- and sex-adjusted	1 (ref)	1.30 (0.62–2.71)	2.18 (1.09–4.37)	0.0143
Multivariate-adjusted ^a	1 (ref)	1.55 (0.73–3.27)	2.42 (1.20–4.91)	0.0088

^a Adjusted for age, sex, duration of diabetes, current smoker, mean SBP, mean HbA1c, mean TC/HDL-C, mean BMI, and the number of SBP measurements (ln-transformed).

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Re-evaluation of patients with type 2 diabetes from the RIACE cohort using the 8th Joint National Committee cut-offs for blood pressure

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Background and aims: The 8th Joint National Committee (JNC8) report has raised the recommended blood pressure (BP) threshold for drug therapy in diabetic subjects from 130/80 (JNC7) to 140/90 mmHg. This study was aimed at re-evaluating with the new BP cut-offs the prevalence of hypertension, anti-hypertensive treatment, and achievement of target BP in patients with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study.

Materials and methods: The RIACE cohort consists of 15,773 patients, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007–2008. Exclusion criteria were dialysis or renal transplantation. BP was measured with a mercury sphygmomanometer on the right arm after at least 5 min of rest. Major acute cardiovascular disease (CVD) events were adjudicated based on hospital discharge records.

Results: Using the JNC7 BP targets, 6,854 patients (43.5%) had systolic BP <130 mmHg, 11,537 (73.1%) had diastolic BP <80 mmHg, and 6,276 (39.8%) met both targets. Using the JNC8 cut-offs, percentages were 66.6% for systolic BP (+23.1%), 94.2% for diastolic BP (+21.1%) and 65.7% for both (+25.9%). Change in percentage of subjects on-target rose from 21.1% to 24.3% for systolic BP and decreased from 25.0% to 16.4% for diastolic BP by age quartiles (p<0.001 for both). Based on JNC7 and JNC8 criteria, respectively, 2,228 and 3,458 patients were normotensive (NT, 14.1% and 21.9%), 13,545 and 12,315 hypertensive (HT, 85.9% and 78.1%), 11,150 and 11,150 treated HT (HT-tx, 70.7% and 70.7%, i.e. 82.3% and 90.5% of HT), and 4,048 and 6,903 on-target HT-tx (HT-tx-target 25.7% and 43.8%, i.e. 36.3% and 61.9% of HT-tx). Then, the following six groups were compared: G1, NT by JNC7 (n. 2,228, 14.1%); G2, NT only by JNC8 (n. 1,230, 7.8%); G3, untreated HT (n. 1,165, 7.4%); G1-tx, on-target by JNC7 (n. 4,048, 25.7%), G2-tx, on-target only by JNC8 (n. 2,855, 18.1%), G3-tx, not on-target (4,247, 26.9%). Prevalence values of any CVD and any coronary event were significantly higher in G1-tx (31.4% and 23.5%, respectively, p<0.001) than in G2-tx (27.6% and 19.6%) and G3-tx (26.3% and 16.1%), likely as an effect of reverse causality, i.e. a higher CVD risk prompted a stronger effort to reduce BP levels.

Conclusion: Achieving BP targets is easier with the JNC 8 criteria. However, it cannot be ruled out that a less aggressive approach might ultimately translate into higher BP levels mainly in subjects at high CVD risk.

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OP 36 Molecular mechanisms of insulin action in vitro

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The regulation of Wnt signalling pathways by insulin and NEFAs in skeletal muscle and adipose tissue of healthy humans

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Background and aims: The Wnt signaling plays an important role in embryonic development, cell proliferation, cell cycle, differentiation, apoptosis and tissue homeostasis. The Wnt ligands bind to frizzled (Fzd) receptors and activate canonical (β -catenin dependent) and non-canonical (β -catenin independent) pathways. Dysregulation of Wnt signal transduction is associated with many pathophysiological states including metabolic disorders. The aim of the present study was to assess skeletal muscle and adipose tissue expression of genes associated with Wnt signaling pathways in young healthy population, their regulation by hyperinsulinemia and serum NEFAs elevation and their relationship with insulin sensitivity.

Materials and methods: We studied 20 healthy male subjects (mean age 25.20 ± 3.15 years, mean BMI 26.47 ± 4.64 kg/m²). The biopsies of vastus lateralis muscle and subcutaneous adipose tissue were performed at baseline and after 6 hours of euglycemic hyperinsulinemic clamp with or without Intralipid/heparin infusion. The participants were divided into subgroups of high (high-IS) and low insulin sensitivity (low-IS).

Results: The muscle mRNA expression of β -catenin, Fzd coreceptor-LDL receptor-related protein 6 (Lrp6), Dishevelled 2 (Dsh2) and adipose tissue mRNA expression of Lrp6 were higher in low-IS, whereas adipose tissue mRNA of Dsh2 and glycogen synthase kinase 3 β (GSK3 β) were lower in low-IS (all $p < 0.05$). Hyperinsulinemia resulted in a decrease in muscle and adipose tissue Lrp6, adipose tissue c-Myc and transcription factor 7-like 2 (TCF7L2) expression and an increase in muscle and adipose tissue Dvl1/2/3 expression (all $p < 0.05$). Intralipid/heparin infusion resulted in 4-fold increase in serum NEFAs ($p < 0.0001$) and decrease in insulin sensitivity by approx. 40% ($p < 0.0001$). Most of the described changes in Wnt signaling pathways disappeared after NEFAs elevation and adipose tissue c-Myc expression was even up-regulated by NEFAs ($p = 0.0005$).

Conclusion: Our data indicate that Wnt canonical signaling is increased in insulin resistant subjects and insulin could inhibit β -catenin dependent pathway and mediate switching between canonical and non-canonical pathways. *Supported by: Grant UDA-POIG.01.03.01-00-128/08; from the Program Innovative Economy*

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Ser312-phosphorylated fetuin-A: role in insulin action and insulin resistance

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Background and aims: Fetuin-A, a hepatokine, inhibits insulin receptor tyrosine kinase and interferes with downstream insulin signaling, and glucose uptake. Elevated levels of fetuin-A has been shown to be correlated with insulin resistance, obesity and type 2 diabetes. Fetuin-A exists in both phosphorylated and dephosphorylated forms in circulation. However, there are no reports on fetuin-A phosphorylation status in insulin resistant conditions or on its molecular characterization. The goal of this study was to characterize the role of fetuin-A phosphorylation on insulin signaling, and its association with obesity and insulin resistance.

Materials and methods: We generated Ser312Ala and Ser120Ala+Ser312Ala mutants to characterize effects of fetuin-A phosphorylation. Ser312-fetuin-A phosphorylation status was examined in diet-induced obese mice, ZDF rats, and obese individuals.

Results: While wild-type phosphorylated fetuin-A inhibited insulin signaling (IR, AKT, and MAPK, GLUT4 translocation), glucose uptake, and glycogen synthesis, the single Ser312Ala-fetuin-A mutant and the double phospho-defective mutant (Ser312Ala+Ser120Ala) were without effect, indicating that phosphorylation status of fetuin-A was critical to mediate its inhibitory effects on insulin action. We next examined phosphorylation status of fetuin-A (Ser312) in animal models of obesity and insulin resistance. Serum Ser312phosphofetuin-A levels were significantly elevated in C57BL/6 diet-induced obese mice and 6 weeks-old ZDF rats, which was consistent with the observed hyperinsulinemia and increased HOMA-IR. Next, we examined total fetuin-A and Ser312-phosphorylated fetuin-A concentrations in 31 obese men and 11 age-matched normal weight individuals. While both serum fetuin-A and Ser312phosphofetuin-A levels were significantly elevated in obese individuals, only Ser312phosphofetuin-A was significantly correlated with serum insulin, HOMA, QUICKI and glucose-to-insulin ratio.

Conclusion: Our studies showing that that phosphorylation status of fetuin-A is critical for its inhibitory effects on insulin action, and that serum Ser312-phosphorylated fetuin-A concentrations are elevated in insulin resistant animals and humans, suggest that phosphorylated fetuin-A plays a key role in the modulation of insulin action.

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Down-regulation of miR-22 and miR-26a independently induces insulin resistance in L6 myotubes

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Background and aims: Insulin resistance (IR) in skeletal muscle invariably develops in advance of type 2 diabetes (T2D). MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate protein translation, via specific binding to the 3'UTRs of mRNAs. The involvement of miRNAs in normal and abnormal physiology is increasingly recognised. However, their role in the development of muscle IR is poorly characterised. We have previously shown the down-regulation of five miRNAs after 5 or 20 weeks of high-fat feeding of male Wistar rats: miR-22, miR-26a, miR-29a, miR-187 and miR-126*. Here we interrogated miR-22 and miR-26a for roles in mediating muscle insulin sensitivity.

Materials and methods: L6 myoblasts were cultured and differentiated into myotubes for study. Firstly, the effects of IR induced by long-term insulin treatment (HI, 10nM), palmitic acid (PA, 0.75mM), TNF- α (2ng/mL) or Dexamethasone (Dex, 2nM) on miRNA expression were investigated. Subsequently, 100nM miR-22 or miR-26a miRNA inhibitor was transfected into myotubes on day 3 of differentiation and the effects on glucose uptake quantified using [H3]-2-deoxy-glucose tracer \pm 100nM insulin. miRNA levels were quantified using probe-based Real-time PCR, normalising to small nucleolar RNA expression. TargetScan was used to predict targets of miR-22 that might impact upon insulin sensitivity and protein levels of these were quantified by western blotting. Data were analysed using Student's paired t-test or Two-way ANOVA with Fisher's LSD test *post-hoc*. Results are presented as percentages of the control or basal treatment condition ($n = 3-4$ independent experiments).

Results: Interestingly, expression levels of both miR-22 and miR-26a in L6 myotubes were reduced by HI (to 74%, $p = 0.047$ and 68%, $p = 0.020$ respectively), but not by PA, TNF- α or Dex treatments, despite impairment in insulin-stimulated glucose disposal. Specific miRNA inhibitor treatment reduced miR-22 and miR-26a expression to 45% $p = 0.0012$ and 41% $p = 0.0002$, respectively. miR-22 inhibition abolished the effect of insulin to stimulate glucose uptake in L6 myotubes, reducing it from 144% of basal in control myotubes to 98% in miR-22 silenced myotubes (Interaction $p = 0.04$). miR-26a inhibition also abolished the effects of insulin stimulation on glucose uptake, although this was principally the result of a 29% increase in basal glucose disposal (Interaction $p = 0.0029$). TargetScan-predicted targets of miR-22 included caveolin-3, sirtuin-1, glucose transporter-1, phosphatase and tensin homolog (PTEN) and protein tyrosine phosphatase, non-receptor type 1. Western blot analysis to date of predicted targets has identified a trend towards increased caveolin-3 (160% $p = 0.11$) after miR-22 silencing, but no effect on PTEN.

Conclusion: These data suggest that hyperinsulinaemia is the key component of the IR syndrome that drives down-regulation of the investigated miRNAs. Both miR-22 and miR-26a are shown to contribute to muscle IR through impairment of insulin-stimulated glucose disposal. The mechanisms for the effects of miRNA inhibition are under investigation.

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GLP-1 improves the reduction of glucose uptake through SIRT-1 in skeletal muscle cells under palmitate induced-insulin resistance

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Background and aims: Insulin resistance is a major pathogenesis of type 2 diabetes mellitus. Recently, the target molecules for diabetes treatment have been identified. Glucagon like peptide-1 (GLP-1) was developed as an anti-diabetic agent and is known to have a potent glucose-dependent insulinotropic action on the pancreas as well as extrapancreatic actions. SIRT1, as an NAD-dependent deacetylase, has been demonstrated in the role of protecting ageing-related diseases and beneficial effects of metabolic homeostasis. We investigated whether the actions of GLP-1 were mediated by SIRT-1 activation in skeletal muscle cells under palmitate induced-insulin resistance.

Materials and methods: After confirming the action of GLP-1 to palmitate-induced insulin resistance, we determined 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NDBG) uptake, GLUT4 mRNA, and the phosphorylation levels of protein related to insulin signal pathway (IRS-1, Akt) in human skeletal muscle myotubes (HSMM) exposed to palmitate (200 µM, for 24 hours) and compared to those in HSMM exposed to palmitate and GLP-1 (200 nM) simultaneously. To elucidate whether SIRT-1 contributed to GLP-1 action in the palmitate-induced insulin resistance, we compared the levels of proliferator-activated receptor-γ-co-activator 1α (PGC1α) deacetylation in HSMM exposed to palmitate and GLP-1 (200 ng/ml) for 24 hours. Moreover, when exposed to SIRT1 inhibitor (EX527) in the same conditions, we demonstrated the changes of GLUT4 mRNA expression and insulin signaling.

Results: GLP-1 restored the reduction of glucose uptake, GLUT4 mRNA levels, and GLUT4 promoter activity by palmitate in human skeletal muscle myotubes. GLP-1 reduced acetylation of PGC1α in HSMMs over expressed PGC1α. This result suggested that GLP-1 activated a deacetylase, such as SIRT-1. SIRT1 inhibitor (EX527) suppressed GLUT4 mRNA expression by GLP-1 in HSMMs. SIRT1 inhibitor (EX527) prevented phosphorylation (IRS-1, Akt) of protein related to insulin signal pathways by GLP-1 in HSMMs. This suggested the expression of GLUT4 mRNA and the activation of insulin signaling by GLP-1 were associated with SIRT1.

Conclusion: Our data suggests GLP-1 improved palmitate-induced insulin resistance in HSMM by the activation of SIRT-1.

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Overexpression of the glucose transporter 1 in renal mesangial cells protects against cellular stress via Nrf2/NQO1

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Background and aims: Diabetic patients have been shown to be susceptible to the formation and accumulation of triose phosphates and the reactive metabolite methylglyoxal (MG), formed by non-enzymatic degradation on these intermediates. These findings suggest that it is not the condition of hyperglycaemia, but how glucose is handled, which underlies the development of diabetic complications. In this project, this was addressed by characterizing rat mesangial cells which stably overexpress the glucose transporter-1 protein, the rate-limiting step for the movement of glucose into the cell. In such cells, glucose uptake and utilization is increased regardless of other regulator factors.

Materials and methods: The phenotype of rat mesangial cells over-expressing glucose transporter-1 (GLUT1) and the respective control cells, stably expressing lac operon (LacZ) were characterized with respect to growth and energy metabolism under basal condition (11mM glucose).

Results: It was found that despite a significantly higher glucose uptake, rat mesangial cells over-expressing glucose transporter-1 (GLUT1) had a reduced glycolytic rate. They also had reduced levels of metabolic cofactors and intermediates. They were also found to have significantly lower number of hyper-polarized mitochondria, suggestive of altered respiratory function. This was confirmed by the decreased rate of oxygen consumption. Mitochondrial superoxide production was shown to be decreased in the GLUT1 cells;

however, generally oxidative stress within these cells was increased, suggestive of increased cellular stress as a consequence of increased glucose uptake. Preliminary screening of antioxidant defence enzymes showed that NAD(P)H dehydrogenase(quinone) 1 (NQO1), was significantly increased in terms of expression and activity as well as its regulator, Nrf2. The observed increase in antioxidant defences was confirmed by the increase in total antioxidant capacity, and increased tolerance to hydrogen peroxide and MG.

Conclusion: This data indicates that under conditions of high glucose uptake, antioxidant defences are increased to help protect the cell from the increased cellular stress. The observed increases in defences system whilst protective are ultimately detrimental to the cell.

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Extracellular vesicles released by hypoxic adipocytes impair insulin signalling and glucose uptake in adipocytes

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Background and aims: Exosomes and microvesicles are extracellular vesicles (EVs) shed by many cell types that mediate cell to cell communication. EVs contain proteins and RNA species that modulate biochemical responses in target cells. Adipocytes have been shown to produce extracellular vesicles. During obesity adipocytes are subject to hypoxia. The aim of our study was to investigate whether EVs released by hypoxic adipocytes have autocrine effects in regulating insulin action and glucose uptake.

Materials and methods: We isolated EVs from the conditioned media of differentiated 3T3L1 adipocytes cultured under normoxia (control cells) or from cells exposed to hypoxia (1% O₂) for 24hrs. EVs were purified from the culturing media using centrifugation techniques and characterized using biophysical and biochemical methods, including nanoparticle tracking analysis, cryoelectron microscopy, and immunoblotting techniques. EVs were then tested for their ability to modulate insulin action and insulin-stimulated glucose uptake in 3T3L1 adipocytes. Independent sets of differentiated 3T3L1 adipocytes were either left untreated or treated with EVs purified from control or hypoxic adipocytes for 24hr and subsequently their response to insulin stimulation was evaluated by western blotting and glucose uptake assays.

Results: We found that 3T3L1 adipocytes release EVs of heterogeneous sizes as determined by cryoelectron microscopy and nanoparticle tracking analysis. Hypoxia increased the release of EVs by adipocytes. Immunoblotting analysis showed that EV preparations were enriched in exosomal markers and markedly devoid of other organelle markers. Adipocytes treated with EVs obtained from hypoxic cells displayed a reduced insulin-mediated activation of glucose uptake compared to those left untreated or treated with EVs derived from control adipocytes. No differences in the expression levels of the glucose transporter Glut4 were observed in cells treated with EVs from hypoxic adipocytes compared to those left untreated or treated with EVs from control cells. However, cells treated with EVs released from hypoxic cells exhibited reduced insulin-mediated activation of phosphatidylinositol 3-kinase as seen by a reduced phosphorylation of the downstream kinase AKT, without alterations in the total AKT levels.

Conclusion: Our data suggest that EVs released by hypoxic adipocytes contribute to insulin resistance, as they impair insulin signalling and glucose uptake in adipose cells.

OP 37 Metformin: new insights into an old drug

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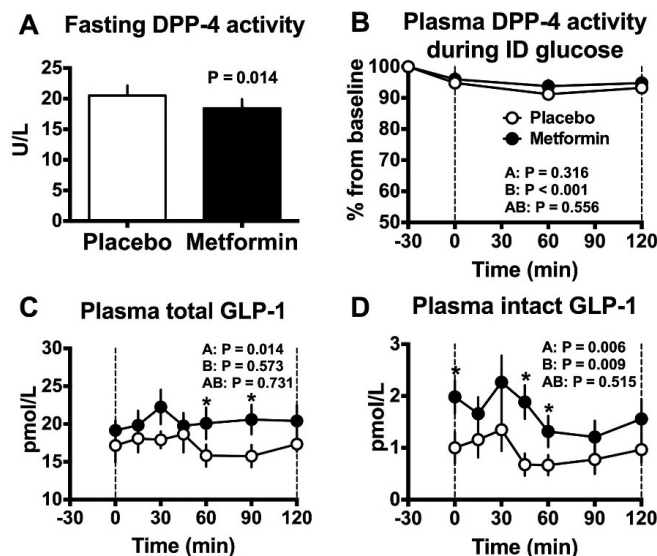
Mechanism of increase in plasma intact GLP-1 by metformin in type 2 diabetes: stimulation of GLP-1 secretion or reduction in plasma DPP-4 activity?

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Background and aims: Metformin increases plasma intact glucagon-like peptide-1 (GLP-1) concentrations. However, it remains uncertain whether this reflects an enhancement of GLP-1 secretion and/or a reduction in plasma dipeptidyl peptidase-4 (DPP-4) activity. We, therefore, evaluated the effects of metformin on plasma DPP-4 activity, and total and intact GLP-1 concentrations, before and during an intraduodenal (ID) glucose infusion in type 2 diabetes.

Materials and methods: We retrospectively assayed plasma DPP-4 activity in 12 Caucasian males with diet-controlled type 2 diabetes (HbA1c: $6.5 \pm 0.1\%$ (47.6 ± 1.4 mmol/mol); duration of known diabetes: 3.5 ± 0.9 years; age: 63.7 ± 1.9 years; BMI: 29.9 ± 1.2 kg/m²), treated with metformin 850 mg bd or placebo each for 7 days in a randomised crossover design, with 14 days 'washout' between. On days 5 or 8 of each treatment (6 patients each), subjects took their morning dose of metformin or placebo after an overnight fast, followed 30 min later by an ID infusion of glucose (60 g over 120 min, ie. 2 kcal/min). **Results:** Plasma fasting DPP-4 activity was less during metformin treatment than placebo (18.4 ± 1.6 vs. 20.5 ± 1.7 U/L, $P = 0.014$). During ID glucose infusion ($t = 0$ –120 min), there was a slight reduction in plasma DPP-4 activity on both days ($P < 0.001$), without any difference between metformin and placebo. Both total and intact GLP-1 concentrations were greater after metformin than placebo ($P = 0.014$ and 0.006 , respectively). During ID glucose infusion, the increment in intact GLP-1 (ie. difference in the areas under the curve (AUC)) after metformin versus placebo was directly related to the AUC for total GLP-1 ($r = 0.61$, $P = 0.028$), and tended to be inversely related to plasma DPP-4 activity ($r = -0.48$, $P = 0.098$).

Conclusion: The increase in plasma intact GLP-1 concentrations by metformin is, at least in part, attributable to stimulation of GLP-1 secretion - a reduction in soluble DPP-4 activity may also make a modest contribution.



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Supported by: MSD funded investigator-initiated study

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Intestinal glucose uptake is modulated by metformin

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Background and aims: Obesity is the result of an imbalance between energy intake and energy expenditure. Recently brown adipose tissue (BAT) has gained a lot of interest because of its capacity to convert calories into heat. BAT shows an intense 18F-Fluorodeoxyglucose-(FDG)-uptake on Positron-Emission-Tomography-(PET)-CT scans. Physiological 18F-FDG accumulation is also frequently observed in the intestine, however, the possibility of the intestine as an energy dissipating tissue has not yet been explored. Metformin is one of the few drugs in the treatment of diabetes mellitus (DM) that is associated with moderate weight loss. Interestingly, patients using metformin are known to have increased 18F-FDG-uptake in the colon. The aim of this study was to retrospectively assess determinants of 18F-FDG-uptake in the intestine in patients who underwent a primary diagnostic 18F-FDG-PET-CT scan.

Materials and methods: Consecutive 18F-FDG-PET-CT scans performed between January 1st and April 31st 2011 were analysed. 18F-FDG intestinal uptake was visually assessed using the 4-point scale described by Gontier et al. which uses liver uptake as a reference. (1; lower, 2; similar, 3; moderately higher than hepatic activity and 4; intense and diffuse uptake). Differences in continuous variables between the 4 grades were assessed using the Kruskal-Wallis test. Differences in categorical variables between the 4 groups were assessed using the Fisher-Freeman-Halton exact test. All variables were used as covariate in a forward logistic regression model (e.g. grade 1 & 2 vs 3&4).

Results: Available for analysis were 270 18F-FDG-PET-CT scans of 270 patients. The majority of patients was female (51.9%) with a median age of 61 [52–71] years, a BMI of $25.0 [22.5–28.7]$ kg/m². Approximately 20% of the subjects smoked (smoking >1 daily). Most patients had a grade 2 (44%) or grade 3 (39%) uptake of 18F-FDG in the intestines, far less subjects had a grade 1 (9%) or grade 4 (8%) uptake of 18F-FDG. There were no significant differences between the four grading groups with regard to age, sex, BMI or smoking status. Type 1 DM was observed in 2 % of the subjects and 37% of the patients had type 2 DM. Of the DM patients, 87.2% used insulin, 87.2% used metformin and 82.1% used sulfonylurea derivatives (SU). There was a positive trend over the 4 grades for the use of insulin, SU and metformin but also for diuretic use. The use of insulin ($p < 0.05$), SU ($p < 0.001$), metformin ($p < 0.001$) and diuretics ($p < 0.05$) was significantly higher in grade 4 than grade 1. In a logistic regression analysis, use of metformin was the only significant predictor for high (e.g. grade 3 or 4 versus grade 1 or 2) 18F-FDG-uptake in all segments of the intestine, with an odds ratio (OR) of 12.4 (correlation coefficient (CC) 2.5, 95%CI 2.8–55.5). However, increased 18F-FDG-uptake in the descendens alone was associated with both metformin with an OR of 17.0 (CC 2.8, 95%CI 5.1–57.4) and anti-inflammatory drugs with an OR of 3.6 (CC 1.2, 95%CI 1.1–11.9).

Conclusion: Metformin use is associated with increased intestinal 18F-FDG-uptake, suggesting a potential role for increasing intestinal energy expenditure.

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Metformin enhances intestinal glucose uptake in patients with type 2 diabetes

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Background and aims: The intestine is emerging as a key organ in the pathogenesis of type 2 diabetes. This study's objective was to determine the effects of metformin and rosiglitazone on intestinal glucose uptake in patients with type 2 diabetes.

Materials and methods: Forty-one patients with newly diagnosed type 2 diabetes were randomized for treatment with metformin (1 g, b.i.d.), rosiglitazone (4 mg, b.i.d.), or placebo in a 26-week double-blind trial. Positron emission tomography (PET) is a key tool for the assessment of intestinal metabolism. Insulin stimulated glucose uptake in small intestine, caecum, and skeletal muscle was measured before and after treatment using [18F]-

fludeoxyglucose (FDG) PET during euglycemic hyperinsulinemic clamp. Magnetic resonance imaging (MRI) was used as an anatomical reference.

Results: Both active treatments improved glycemic control ($p < 0.01$ vs. placebo). Rosiglitazone improved glucose uptake by 44% in the whole body ($p < 0.05$) and by 38% in skeletal muscle ($p < 0.01$, in both groups), while no changes were observed in the metformin group as previously reported (Diabetes 2002). In pooled data small bowel and caecal GU were associated with fGluc ($r = -0.52$, $p = 0.001$; $r = -0.43$, $p = 0.01$) and HbA1c ($r = -0.40$, $p < 0.02$; $r = -0.46$, $p = 0.005$). Glucose uptake was increased 2-fold in the metformin group in small intestine (2.0 ± 0.7 vs. 4.3 ± 1.6 μmol [100 g](-1) min(-1), $p < 0.0001$) and by 3.7-fold in caecum (1.6 ± 0.8 vs. 5.0 ± 1.9 μmol [100 g](-1) min(-1), $p < 0.0001$). A significant but minor increase in the rosiglitazone group occurred in the small intestine (2.3 ± 0.6 vs. 2.8 ± 0.8 μmol [100 g](-1) min(-1), $p < 0.03$) and this increment was associated with M-value ($r = 0.67$, $p < 0.02$). No changes in intestinal glucose metabolism were observed in the placebo group.

Conclusion: This study shows that metformin enhances glucose metabolism both in the small intestine and the large intestine in patients with newly diagnosed type 2 diabetes. This effect is not related to enhanced insulin sensitivity but associates with improved glycemic control.

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Tissue hypoxic condition, not metformin, induces lactic acidosis in patient type 2 diabetes

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Background and aims: Metformin is recommended as the first drug in type 2 diabetic patients and has been shown to reduce diabetic-related complications and mortality. However, the use of metformin is limited by a concern of lactic acidosis (LA), especially in patients with renal failure. Therefore, we investigated the lactic acidosis and its relationship with metformin use in patients with type 2 diabetes.

Materials and methods: A total of 1,954 patients with type 2 diabetes were recruited from 2007 to 2011. They were analyzed according to the estimated glomerular filtration rate (eGFR) of 60 mL/min/1.72m². LA was defined as serum lactate levels more than 5 mmol/l and arterial pH less than 7.35.

Results: According to the metformin use, there were no differences in serum lactate levels in patients with type 2 diabetes. In addition, serum lactate levels were not correlated with metformin dose ($P = 0.350$). These results were comparable in patients with eGFR < 60 mL/min/1.72m². Overall, the prevalence of hyperlactinemia and LA were 18.8% and 3.0%, respectively. In subgroup analysis, they showed similar results regardless of metformin use. All patients with LA had at least one condition related to hypoxia or poor tissue perfusion such as myocardial infarction, septic shock, advanced cirrhosis and bleeding. On multiple regression analysis, metformin did not affect the development of lactic acidosis. Meanwhile, the tissue hypoxic condition was an independent risk factor for the development of lactic acidosis (odds ratio: 4.437, 95% confidence interval: 1.277–15.425).

Conclusion: In conclusion, tissue hypoxic condition was the one of the most attributable factors for the development of lactic acidosis in patients with type 2 diabetes. The metformin use may not be the cause of lactic acidosis, but rather a coincidence in patients with type 2 diabetes.

OP 38 Novelties in risk prediction

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How can we best predict cardiovascular disease risk in type 1 diabetes?

A comparison of risk equations

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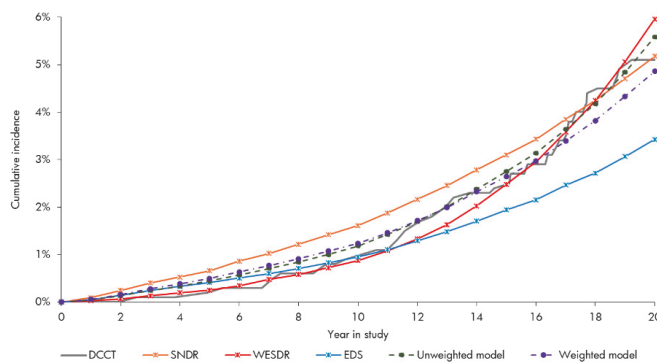
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Background and aims: Patients with type 1 diabetes (T1D) are at increased risk of developing cardiovascular disease (CVD) compared with the general population. In this study, the generalizability of CVD prediction models for patients with T1D, as well as a model averaging approach, is evaluated.

Materials and methods: Literature review identified two models of CVD risk and three clinical studies in patients with T1D that could be used to derive de novo formulae for estimating CVD risk. The five models of CVD were derived from the: EuroDiab study (EDS), FinnDiane Registry (FDR), Lung model (LM, based on Diabetes Control and Complications Trial [DCCT] data), Swedish National Diabetes Registry (SNDR), and Wisconsin Epidemiologic Study of Diabetic Retinopathy study (WESDR). Two additional models were developed via model averaging. The first used the mean event probability over all models to define the patient's risk of CVD. The second applied a weight, calculated as the sum of log ratios of six cohort characteristics, to each model. Prediction performance was evaluated between models and against first incident CVD in the DCCT, defined as myocardial infarction (MI), stroke or unstable angina. Two algorithms, FDR and LM, only estimated risk of MI and stroke. Goodness of fit was assessed using the Chi squared test. A successful prediction was defined as $\geq 60\%$ likelihood ($p \geq 0.6$) that the two distributions (model versus actual) were not significantly different.

Results: Comparing the predictive performance of the three models that estimated all three CVD endpoints, CVD events in the DCCT were successfully predicted by each model, although generally SNDR overestimated and EDS underestimated risk (Figure). WESDR ($p = 0.99$) most closely matched outcomes in the DCCT. Both model averaging approaches (unweighted and weighted) made successful predictions (both $p = 0.99$). Inter model comparison showed that no individual model could successfully predict outcomes in more than two of the other datasets. The unweighted model average did not aid prediction performance, however, the weighted model made successful predictions in all patient populations: SNDR ($p = 0.99$), EDS ($p = 0.6$), FDR ($p = 0.6$), WESDR ($p = 0.99$), LM ($p = 0.99$) and DCCT ($p = 0.99$).

Conclusion: A model averaging approach weighted to baseline patient characteristics was the only model to provide a successful prediction in all six test cases. By evaluating multiple risk factors, model averaging may have advantages over traditional approaches to estimating CVD risk in patients with T1D.



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Occurrence of clinical remission in adult-onset type 1 diabetes is associated with decreased risk of microvascular complications

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Background and aims: Partial clinical remission is common in the initial course of type 1 diabetes. In this phase of disease substantial insulin secretion contributes to good metabolic control and low incidence of acute complications. The aim of the study was to determine the association between presence of partial remission and occurrence of chronic complications of type 1 diabetes.

Materials and methods: 240 consecutive patients (77 women, 143 men), aged 26 (IQR: 22–31) hospitalized with newly diagnosed type 1 diabetes in the Department of Internal Medicine and Diabetology in 2004–2007 were asked to participate. Of these, 220 were included in the study. 133 of patients completed prospective follow-up. Finally, data of 81 patients (24 women, 57 men), aged 33 (IQR 29–38) were included into endpoint analysis. Clinical remission was defined as time in which all of the following criteria were met: HbA1c below 6.5 %, dose of exogenous insulin below 0.3 U / kg body weight and serum C-peptide concentration above 0.5 ng / ml. Patients were divided into those who were in remission at any time during follow-up (remitters) and non-remitters. At 7 years of follow-up occurrence of chronic microvascular complications of diabetes (retinopathy, diabetic kidney disease and neuropathy) was evaluated.

Results: In non-remitters group higher incidence of at least one microvascular complication (46.4 vs. 7.6 %, $p < 0.0001$), higher incidence of retinopathy (42.8 vs. 5.7 %, $p < 0.0001$), and neuropathy (21.4 vs 1.9 %, $p = 0.006$) was found. In univariate logistic regression, significant association was found between absence of remission and occurrence of at least one microvascular complication (OR: 10.6, 95% CI : 2.94–38.22, $p = 0.002$). In the Cox proportional hazards regression model that included clinically significant parameters at diagnosis (presence of ketoacidosis, cigarette smoking and HbA1c value) as covariates, absence of remission was associated with occurrence of chronic complications of diabetes at 7 years [HR: 3.65 (95% CI 1.23–4.56), $p = 0.04$].

Conclusion: Occurrence of clinical remission of diabetes is associated with reduced risk of chronic microvascular complications at 7-year follow-up.

Supported by: The Polish Diabetes Association

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Proinsulin in early atherosclerosis: causal factor or bystander?

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Background and aims: Increased proinsulin levels (relative to insulin levels) have been demonstrated to predict future incidence of both Type 2 Diabetes (T2D) and cardiovascular disease (CVD), independently of classical risk factors, although the mechanisms of these relationships are poorly understood. A genome-wide association meta-analysis study conducted by the MAGIC consortium, and including this group, has identified a number of loci and genetic variants associated with increased circulating proinsulin levels. This project aims to determine whether proinsulin and/or proinsulin-associated pathways play a causal role in determining severity and rate of progression of early, subclinical atherosclerosis.

Materials and methods: The IMPROVE study (consisting of 3711 subjects, of whom 917 are T2D subjects) without clinical signs of CVD at enrolment, who have been carefully phenotyped and monitored with repeated state-of-the-art high-resolution carotid ultrasound examinations. Biochemical phenotyping includes measurement (by Elisa) of circulating insulin and proinsulin levels. All subjects have been genotyped on the Illumina CardioMeta200K platform, providing good coverage of 5 of the 9 loci associated with proinsulin levels. Additional genotyping to cover the remaining 4 loci has been performed. Standard statistical methods and a Mendelian randomisa-

tion approach will be used to examine the influence and causality of proinsulin on early atherosclerosis.

Results: Proinsulin levels demonstrated associations with progression of (but not baseline) carotid intima media thickness (cIMT). In contrast, proinsulin-associated genetic variants influenced baseline but not progression cIMT measures, independently of established CVD risk factors and proinsulin levels. Novel associations between a) novel genetic variants and proinsulin and b) proinsulin-associated SNPs and cIMT have been observed, however these require replication.

Conclusion: Proinsulin does not appear to be a causal factor in early sub-clinical atherosclerosis, however proinsulin-associated mechanisms are implicated.

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Risk of cardiovascular disease events: the impact of diabetes and anti-diabetic drugs: a nested case-control study

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Background and aims: Diabetes Mellitus (DM) is associated with an increased risk of cardiovascular disease. We investigated the effects of antidiabetic drugs on the composite endpoint (CE) of ischemic heart disease, heart failure and stroke incidence in patients with DM.

Materials and methods: We conducted a nested case-control study. Cases were patients with type 1 or type 2 DM who subsequently suffered from CE; controls were DM patients with no history of CE after DM diagnosis. Using the Danish National Hospital Discharge Register, we included DM patients with information on date of DM diagnosis, date of CE, and comorbidities. From the Central Region of Jutland, Denmark, medication use and biochemical parameters (LDL, HDL, total cholesterol, triglyceride, HbA1c, and creatinine) were collected. Logistic regression analyses were conducted. The analyses were mutually adjusted for comorbidities, pharmaceutical use, and the biochemical parameters.

Results: The data included 14,454 patients with DM who contributed a total of 115,225 person-years. 14.8 percent (2,257) suffered from a subsequent CE. CE prior to DM diagnosis (OR= 14.83, 95%CI: 12.63–17.41), atrial fibrillation (OR= 1.99, 95%CI: 1.57–2.51), and age at diabetes diagnosis (OR= 1.01, 95%CI: 1.007–1.014) all significantly increased the risk of subsequent CE. Patients with type 2 DM had a higher risk compared to type 1 DM patients (OR= 3.05, 95%CI: 2.57–3.63). The diabetes complications retinopathy (OR= 1.70, 95%CI: 1.30–2.23), nephropathy (OR= 1.58, 95%CI: 1.28–1.95), neuropathy (OR= 1.99, 95%CI: 1.57–2.53) and peripheral artery disease (OR= 2.06, 95%CI: 1.68–2.56) increased the risk of CE so did male gender (OR= 1.12, 95%CI: 1.00–1.25), and hypertension (OR= 1.39, 95%CI: 1.20–1.62). Treatment with statins reduced the risk of CE significantly (OR= 0.65, 95%CI: 0.57–0.73) when looking at the DM group as a whole, when grouped only significant in subjects with type 2 DM (OR= 0.59, 95%CI: 0.51–0.67). Biguanides (OR= 0.54 95% CI: 0.44–0.67) and liraglutide (OR= 0.41 95% CI: 0.32–0.52) (type 2 DM) both significantly decreased the risk of CE. DPP-4 inhibitors and β -cell stimulating agents had a neutral effect. In the subgroup analysis, a total of 1,351 DM patients (10,229 person-years), had information on biochemical values and were included. 14.9 percent (201) suffered from a subsequent CE. When results were adjusted for biochemistry, liraglutide (OR= 0.25, 95%CI: 0.11–0.56) was the only antidiabetic drug that retained a significant reduction on the risk of CE.

Conclusion: We have shown an association between use of biguanides and liraglutide and a reduced risk of CE in patients with type 2 DM. The effect of liraglutide was not tied to patient biochemical values, e.g. cholesterol or glucose-control (HbA1c).

Supported by: Novo Nordisk

OP 39 Inflammation in obesity and type 2 diabetes

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Activation of oestrogen receptor alpha in macrophages controls high-fat diet-induced inflammation of adipose tissue and prevents obesity and glucose intolerance

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Background and aims: Estrogens have been recognized as key regulators of body composition and glucose homeostasis. However, although both clinical and experimental data clearly evidenced the crucial role of estrogen receptor alpha (ERα), the mechanisms involved in the protective actions of estrogens against obesity and diabetes remain obscure. For instance, although estrogens are known to modulate inflammatory responses through ERα dependent effects, it is still uncertain whether specific actions of these sex steroid hormones on the stroma-vascular fraction (SVF) of adipose tissue could contribute to their benefits on body composition and glucose metabolism. In the present study, we aimed to determine 1) the influence of estrogens on the adaptation of SVF cells in response to a nutritional stress; 2) the contribution of ERα-expressing myeloid cells to the prevention of obesity by estrogens.

Materials and methods: In a first set of experiment, 3 groups of C57Bl/6 female mice were subjected to a chow or a high-fat (HFD) diet for 12 weeks: ovariectomized (estrogen deficiency), sham-operated (endogenous estrogens) and ovariectomized treated with 17β-estradiol (E2, 80μg/kg/d, sc). Weight gain, body composition and glucose tolerance were monitored and both isolated adipocytes and SVF from visceral adipose tissue (VAT) were analyzed by flow cytometry and RT-qPCR. Then, metabolic phenotype and SVF characteristics were studied in HFD-fed mice with specific invalidation of ERα in myeloid cells (ERα-LysM-Cre+ mice).

Results: Both endogenous estrogens and E2 administration prevented HFD-induced obesity and glucose intolerance, as well as adipocyte hypertrophy. HFD increased the number of CD45+ cells in VAT SVF, irrespective of estrogen status, but VAT infiltration by immune cells (CD45+) was strongly reduced by endogenous estrogens and E2, with a significant decrease in macrophage content (~65% CD45+/F4/80+ cells) and a predominance of M2 macrophages, as confirmed by mRNA analysis (+13-fold Ym1; -4.5-fold iNOS in E2-treated as compared to ovariectomized mice). VAT infiltration by B and TCD4+ and TCD8+ lymphocytes was also reduced by estrogens, but the pool of regulatory T cells was preserved. Demonstrating the role of ERα-LysM-Cre+ mice were characterized by a significant increase of HFD-induced inflammation of VAT and developed exacerbated adiposity and glucose intolerance.

Conclusion: These data demonstrate that estrogens limit HFD-induced inflammation of VAT and suggest that macrophages of the SVF largely contribute to their protective action against obesity and through the activation of ERα.

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Heme oxygenase-1 drives metaflammation and insulin resistance in mouse and man

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Background and aims: Obesity and diabetes affect more than half a billion individuals worldwide. Interestingly, the two conditions do not always co-incide and the molecular determinants of 'healthy' versus 'unhealthy' obesity remain ill-defined. Chronic metabolic inflammation (metaflammation) is believed to be pivotal. Here, we tested a hypothesized anti-inflammatory

role for heme oxygenase-1 (HO-1) in the development of human and mouse metabolic disease. Heme oxygenases catalyze the oxidative degradation of heme, a potentially harmful pro-oxidant, into biologically active products: biliverdin IXα, carbon monoxide(CO) and ferrous iron. In addition to its role in heme catabolism, HO-1 plays important roles in various pathophysiological states associated with cellular stress.

Materials and methods: To address the metabolic role of HO-1 in vivo in macrophages, we have generated a myeloid-cell specific conditional HO-1 knockout mouse model ('MacHO') and examined the role of HO-1 in the development of obesity-associated adipose tissue inflammation and insulin resistance. We fed either low-fat (10% calories derived from fat) or high-fat (60% of calories derived from fat) diets to male MacHO and littermate control mice for a total of 21 weeks.

Results: A significant difference in body-weight between the high fat diet groups was observed after 8 weeks of HFD feeding. Both insulin sensitivity and glucose tolerance were improved in MacHO mice fed the HFD compared with littermate control mice. Similarly, obese MacHO mice showed significantly higher insulin sensitivity as measured by insulin tolerance test when compared to HFD-fed control-mice, whereas insulin sensitivity was comparable between both groups on control diet. Histological analysis of HFD VAT sections showed a higher density of smaller adipocytes and a significant reduction in macrophage cell numbers, especially of the Cd11c+ pro-inflammatory macrophage subpopulation. Furthermore, we found that mice lacking myeloid Hmox1 exhibited a strong reduction in hepatic accumulation of triglycerides and cholesterol. Histological analysis showed decreased vacuole formation and a reduced number of lipid droplets, consistent with amelioration of hepatic steatosis. In line, in highly matched biopsies from 'healthy' versus insulin resistant obese subjects we find HO-1 to be amongst the strongest positive predictors of metabolic disease in humans.

Conclusion: Intriguingly, cellular assays show that HO-1 is defining pre-stimulation thresholds for inflammatory skewing and NF-κB amplification in macrophages. These findings identify HO-1 inhibition as a potential therapeutic strategy for metabolic disease.

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Adipose stem cells contribute to inflammation and insulin resistance through both TH-17 polarisation and increased pro-inflammatory cytokine secretion

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Background and aims: In obesity, white adipose tissue (WAT)-infiltrating immune cells induce a state of low grade inflammation. Among those cells, Th-17 cells have been identified as contributors of WAT inflammation. However, the mechanisms by which Th-17 are activated remain to be elucidated. Therefore, to gain further insight into the inflammatory processes taking place into WAT during obesity, we investigated whether adipose tissue-derived stem cells (ASC) could promote Th-17 cell activation.

Materials and methods: Human ASC were isolated from the stromal vascular fraction of residual WAT obtained during supra-mesocolic abdominal surgical procedures. Mononuclear cells (MNC) were prepared from blood donors, or from three surgical patients, as autologous controls. Co-cultures were performed with MNC and graded doses of ASC. Phyto-hemagglutinin A (PHA) was used for MNC stimulation. Cytokine secretion and mRNA expression were measured by ELISA and quantitative PCR, respectively. Binding of STAT3 and STAT5 on the IL-17 genomic locus was measured by chromatin immunoprecipitation (ChIP) in co-cultured cells. Finally, adipogenesis was induced in the presence or absence of conditioning medium collected from ASC-MNC co-cultures. Insulin-sensitivity and adipocyte differentiation were measured through AKT phosphorylation, and expression of mRNA markers, respectively.

Results: Co-cultures of ASC and PHA-activated MNC resulted in a dose-dependent increase of IL-17A secretion (p<0.0001, at the highest ASC: MNC ratio, as compared with no ASC), and in an increase of IL-1b and IL-6 secretion. In contrast, TNFα and IL-2 decreased (p=0.0003, and 0.0116, respectively), suggesting polarization towards the Th-17 lineage, and reduction of the Th-1 cell subset. ASC induced an increase of STAT3 binding on the IL-17 locus, as measured by ChIP. Physical contact between ASC and MNC

played an important role in ASC-mediated enhancement of IL-17A production, as co-cultures in transwell systems drastically decreased IL-17A production ($p < 0.01$). Finally, when conditioning medium of co-cultured cells was added to fresh ASC during their differentiation into adipocytes, a reduction in insulin-mediated AKT phosphorylation was observed, together with a potent inhibition of adipogenesis, as assessed by decreased mRNA expression of FABP-4, adiponectin and PPAR γ .

Conclusion: Our data demonstrate that in response to physical interaction between ASC and MNC, ASC mediate (i) polarization of T cells towards the Th-17 pathway, and (ii) enhancement of IL-1b and IL-6 secretion. Furthermore, such ASC-mediated cytokine secretion impairs ASC adipogenic capacity and adipocyte insulin sensitivity. Thus, our data suggest that ASC from WAT might contribute to the initiation of low-grade chronic inflammation in obese patients through increased secretion of IL-17A and pro-inflammatory cytokines, which may in turn inhibit adipocyte differentiation.

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Interleukin-15 signalling promotes high fat diet-induced inflammation in the liver

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Background and aims: Interleukin-15 (IL-15) is essential for the homeostasis of lymphoid cells particularly memory CD8 T cells and NK cells. These cells are abundant in the liver, and are implicated in obesity-associated pathogenic processes. Our recent findings show that IL-15 knockout mice are resistant to high fat diet-induced obesity. IL-15 is unique among the common gamma chain receptor family of cytokines in that it needs to be trans-presented by the IL-15 receptor alpha chain (IL-15Ra). The goal of this study is to characterize obesity-associated metabolic and cellular changes in the liver of mice lacking IL-15 or IL-15Ra.

Materials and methods: Wildtype, IL-15 knockout (IL15-KO) and IL-15Ra KO (IL15Ra-KO) mice in C57BL/6 genetic background were maintained on high fat diet (HFD) or normal control diet. After 16 weeks, liver mass, fat accumulation in the liver, serum lipid levels and gene expression in the liver were evaluated. Primary hepatocytes were stimulated with IL-15, and signaling and gene expression were studied. Intrahepatic lymphocytes (IHL) were examined in WT, IL15-KO and IL15Ra-KO mice, as well as in hepatocyte-specific IL15Ra-KO mice by flow cytometry.

Results: Diet-increase in liver weight and accumulation of lipids in the liver are prevented by IL-15 or IL-15Ra deficiency. IL-15 deficiency does not affect diet-induced expression of enzymes involved in beta-oxidation of lipids in the liver. However, the liver tissues of IL15-KO and IL15Ra-KO mice showed decreased expression of TNF α and iNOS, chemokines CCL2, CCL5 and CXCL10, and macrophage markers CD68 and F4/80. IL-15 stimulation induced chemokine gene expression in wildtype and IL15-KO hepatocytes, but not in IL15Ra-KO hepatocytes. High fat diet enhances the number of CD4, CD8, NK and NKT cells infiltrating the liver. The NK and NKT subsets in intrahepatic lymphocytes are severely reduced in mice lacking IL-15Ra.

Conclusion: High fat diet-induced lipid accumulation in the liver is mediated, at least partly, by IL-15. High fat diet induces IL-15 gene expression in the liver and this is associated with increased inflammatory response. IL-15 induces chemokine gene expression in hepatocytes that may account for increased intrahepatic lymphocytes. Overall, our findings support the idea that increased availability of IL-15 in obesity may stimulate hepatocytes to promote inflammation in the liver, leading to fatty liver disease.

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OP 40 Translational immunology of type 1 diabetes

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Functional genomics identifies *tnfaip3/A20* as a diabetes protective gene

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Background and aims: Single-nucleotide polymorphisms (SNPs) within *tnfaip3* (also known as A20) associate with susceptibility to type 1 diabetes and complications in type 2 diabetes. How polymorphisms within the *tnfaip3/A20* locus contribute to disease at the islet level is not understood.

Materials and methods: In a genome-wide *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis screen of C57BL/6 mice we identified a mouse line harbouring a non-synonymous amino-acid change, at an evolutionary conserved isoleucine. The T>A mutation substituted asparagine at residue 325 in place of isoleucine (A20^{I325N}) that lies in a highly conserved beta-hairpin loop in A20s ovarian tumour (OTU) domain, away from the C103 catalytic residue. Despite the presence of increased circulating granulocytes and CD44^{high} T-cells, adult mice appear grossly normal and healthy; we therefore tested the effect of the mutation on islet biology.

Results: Here we demonstrate that pancreatic islets harbouring the A20^{I325N} mutation to exhibit cell-intrinsic increased susceptibility to inflammatory triggers, with loss of metabolic function. When challenged with mild inflammatory stress by syngeneic transplantation of A20^{I325N} islets into wildtype (WT) hosts, A20^{I325N} islet grafts showed an abnormal production of inflammatory factors (e.g. CXCL10, CXCL1) with significant neutrophil infiltration. Furthermore, mutant islets exhibited severe glucose intolerance in an i.p.GTT, and an i.v.GTT revealed that beta-cell function was impaired. When directly challenged with an immunological insult in the form of allogeneic transplantation, A20^{I325N} islets were hyper-inflammatory and more rapidly destroyed than WT allogeneic islets. Immunoblot analysis of cells harboring the A20^{I325N} mutation show exaggerated poly-ubiquitination of RIP1 with exacerbated NF- κ B and JNK/AP-1 signaling following TNF-stimulation. In vitro transfection studies using NF- κ B and AP-1 luciferase reporter plasmids show WT A20 to inhibit luciferase production at the promoter level. Thus, A20 is critical for controlling NF- κ B but also JNK activation; and loss of OTU function results in tissue hypersensitivity to inflammatory triggers and metabolic impairment. Ectopic expression of WT A20 in A20^{I325N} islets restored control of NF- κ B and JNK/AP1 pathways and rescued A20^{I325N} islets from hyper-inflammation. Whole body A20^{I325N} mutant mice show normal glucose homeostasis, however, in a diet induced obesity model (45 kcal % fat), mice homozygous for the point mutation (A20^{I325N/I325N}) develop more severe glucose intolerance, compared to littermate controls and mice heterozygous for the point mutation (A20^{I325N/+}). However, when A20^{I325N/+} mice are subjected to a low-dose lipopolysaccharide (LPS; i.p.) challenge, they also developed marked glucose intolerance compared to littermate WT controls. Furthermore, glucose intolerance was coupled with islet-specific immune infiltration of the pancreas.

Conclusion: These data identify A20 as a diabetes protective gene. A20 negative feed-back on NF- κ B and JNK/AP-1 pathways maintain islet inflammatory and metabolic homeostasis under vigorous inflammatory states. In contrast, functional impairment by SNPs can uncouple A20s protective effect and lead to diabetogenic outcomes.

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Anti-CD3 / anti-CXCL10 antibody combination therapy reverts type 1 diabetes in two mouse models

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Background and aims: Therapy of type 1 diabetes (T1D) with anti-CD3 monoclonal antibodies (a-CD3) results in a blockade of the autoimmune process in both animal models and patients with T1D patients. Treated patients show a reduced insulin need and elevated C-peptide levels. Unfortunately, this effect is only temporal and patients revert within a few years to the status they had at the beginning of the therapy. In animal models several combination therapies using a-CD3 and treatments, such as rapamycin, nasal insulin, vitamin D, FTY 720, or *Lactococcus lactis* have been successful in

blocking T1D. We used a different approach and aimed at a blockade to cellular re-entry into the islets of Langerhans after a-CD3 treatment by blocking the key chemokine CXCL10.

Materials and methods: We used the well-established RIP-LCMV-GP mouse model of T1D. As a target autoantigen in the β -cells, such mice express the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) under control of the rat insulin promoter (RIP). RIP-LCMV-GP mice only develop T1D only after infection with LCMV. For the present study we used a combination therapy (CT) and treated diabetic RIP-LCMV-GP mice first with a-CD3 (3 injections in 3 days) followed by administration of a neutralizing anti-CXCL10 monoclonal antibody (a-CXCL10) (8 injections over 3 weeks). **Results:** The CT reverted T1D in RIP-LCMV-GP mice significantly (CT: 65% reversion; $p=0.0078$ / Control: 20% reversion). Thereby, the efficacy of the monotherapies was improved (a-CD3 alone: 47% reversion; $p=0.1168$ / a-CXCL10 alone: 36% reversion; $p=0.3735$). Flow cytometry and histological experiments demonstrate a marked reduction of CD4 and CD8 T cells in the pancreas of mice treated with the CT compared to mono-therapies at day 31 after infection. Importantly, the presence of islet antigen (LCMV-GP)-specific CD8 T cells was reduced dramatically. This effect is long-lasting since CT-treated islets show almost no infiltrates up to 181 days after infection. In order to confirm our data in a different model, we conducted a pilot study with NOD mice, in which CT cured 50% of diabetic mice whereas no mice show diabetes reversion with a-CD3 or without therapy.

Conclusion: In summary our data suggest that a combination therapy of anti-CD3 and anti-CXCL10 antibodies results in a significant reduction of T1D in two independent mouse models. The neutralization of the key chemokine CXCL10 prevented a re-entry of aggressive T cells into the pancreas and thereby abrogated the destruction of beta-cells. Such a combination therapy might constitute a novel treatment for patients suffering from T1D.

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MiR-125a-5p is up-regulated in un-functional CD4+FOXP3+ T regulatory cells deriving from pancreatic lymph nodes of patients with type 1 diabetes and targets C-C chemokine receptor type 2
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Background and aims: We previously showed that CD4+CD25brightCD127- T regulatory (Treg) cells deriving from pancreatic lymph node (PLN) of patients with type 1 diabetes (T1D) are epigenetically imprinted to be Treg, but for unknown reasons do not function as such in vitro. Importantly, this functional defect is present only in Treg cells residing in the PLN of T1D patients and not in those circulating in their peripheral blood (PB). Some microRNAs (miRNAs), an abundant class of small non-coding RNAs that regulate gene expression by affecting the degradation and translation of target mRNAs, are differentially expressed in Treg cells isolated from PLN as compared to those isolated from PB of T1D patients. Among the differentially expressed miRNAs, we found that miR-125a-5p is specifically up-regulated in Treg cells isolated from PLN of T1D patients as compared to those isolated from PB of the same donors and to those isolated from non-diabetic donors. This differential expression was not identified in T conventional cells of T1D patients, uncovering both a cell (i.e., Treg cells) and a disease-specific (i.e., T1D) miRNA expression. Thus, we aimed at further investigating the role of miR-125a-5p in Treg cells.

Materials and methods: CD4+CD25brightCD127- Treg cells were FACS sorted from PLN and PB of patients with T1D and from non-diabetic donors. 200 sorted cells were analyzed and miRNA expression was evaluated by RT-PCR using Taqman miRNAs single assay. Data analysis was performed using 2-ddCt method. Bioinformatic miRNAs target prediction was performed using Targetscan 6.2. Dual luciferase reporter assay was performed to experimentally validate the predicted target gene. CCR2 expression was determined by flow cytometry.

Results: Computational target gene analysis of miR-125a-5p was performed. Target prediction analysis retrieved some potential interesting genes involved in Treg-cell biology, including: C-C chemokine receptor type 2 (CCR2), interleukin 6 receptor (IL6R), interferon regulatory factor 4 (IRF4). Among these putative targets we focused on CCR2 to verify whether this chemokine receptor is key in the observed Treg-cell dysfunction. Firstly, this prediction was validated by renilla luciferase assay: a decreased luciferase activity following

miR-125a-5p overexpression was observed and this reduction was restored upon miRNA predicted target site mutations. Secondly, CCR2 expression on Treg cells was tested by flow cytometry and frequency of CCR2+ Treg cells was correlated with miR-125a-5p RT-PCR levels. Interestingly, lower expression of CCR2+ Treg cells correlated with higher expression of miR-125a-5p, suggesting an inverse correlation.

Conclusion: A disease- and tissue-specific miRNA, miR-125a-5p, has been identified in Treg cells isolated from PLN of patients with T1D. miR-125a-5p targets CCR2, thus leading to reduced CCR2 expression. Reduced expression of CCR2 may induce migration dysfunction in Treg cells residing in the PLN of T1D patients. Therefore these Treg cells could be unable to reach the target organ where they are supposed to control and possibly block the autoreactive immune response. Further in vitro and in vivo studies are ongoing to confirm this hypothesis.

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Cholecalciferol increases suppressor function in regulatory CD4+CD25+Foxp3+ T-cells from patients with new onset type 1 diabetes: a randomised controlled trial

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Background and aims: Vitamin D has an important role in increasing the effects of innate immune processes while restraining the adaptive immune system, leading to improved outcomes in autoimmune diseases. Vitamin D deficiency is one environmental factor to influence the risk of type 1 diabetes (T1D) and in vitro data reinforce the role of vitamin D in the pathogenesis of T1D. In this study we evaluate the effects of Cholecalciferol on function of regulatory T-cells and residual b-cell function in patients with recent onset T1D.

Materials and methods: In this randomized, double-blinded, placebo controlled trial thirty patients (age 15 [10-16], 77% male) with new-onset T1D were assigned to receive oral therapy of cholecalciferol (70IU/kg bodyweight/day) or placebo for 12 months. Cellular frequencies were determined by FACS-analysis and functional tests were assessed with ex vivo suppression co-cultures. Mixed meal tolerance tests were performed to assess b-cell function.

Results: Suppressive capacity of regulatory T cells (Treg) increased with Cholecalciferol after 3, 6 and 12 month ($p=0.0005$) and delta suppression capacity between Cholecalciferol (28.1 [14.9 to 43.3]%) and placebo (-20.9 [-30.1 to -11.3]%) was significant ($p=0.002$). Treg frequency did not differ after 12 month between the two groups (5.75 \pm 1.48% vs 5.38 \pm 1.73%, $p=0.187$). HbA1c and insulin dose were similar between the groups, fasting C-peptide appeared to decrease slower ($p=0.078$) in the Cholecalciferol group (0.62 \pm 0.18 to 0.59 \pm 0.34 ng/ml) than in placebo group (0.65 \pm 0.27 to 0.40 \pm 0.25 ng/ml). Serum calcium and parathormone stayed within the normal range.

Conclusion: Cholecalciferol, the inactive form of vitamin D used as adjunctive immunomodulatory therapy with insulin is associated with an improvement in suppressor function of regulatory T-cells in patients with new-onset T1D. Assessment of vitamin D status and a targeted supplementation may be indicated after the diagnosis of T1D.

Clinical Trial Registration Number: NCT01390480

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OP 41 The human methylome in diabetes

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Novel epigenetic markers of type 2 diabetes in the liver

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Background and aims: The liver plays an important role in the regulation of glucose homeostasis. Epigenetic markers in this organ could contribute to the pathophysiology and heritability of type 2 diabetes (T2D). Our aims were to define DNA methylation patterns associated with T2D risk in the liver, correlate these epigenetic factors with gene expression pathways and decipher intermediate metabolic traits.

Materials and methods: We analyzed the genome-wide methylation patterns in the liver tissue of 96 T2D cases and 96 matched normoglycemic controls using Infinium Human Methylation 450 BeadChips (Illumina). A beta-mixture quantile normalization method was applied for correcting probe design bias. Genome-wide expression profiles were also assessed in a subset of 24 T2D cases and 24 matched normoglycemic controls using HumanHT-12 v4 Expression BeadChips (Illumina).

Results: We identified 158 sites differentially methylated between T2D cases and controls after Bonferroni correction for multiple comparisons ($p < 1.5 \times 10^{-7}$). Taken together, these sites tended to be hypomethylated in T2D cases compared to normoglycemic controls ($p = 1 \times 10^{-7}$). Only two of these cytosine methylation marks were located near functionally relevant candidate genes differentially expressed between T2D cases and controls ($p < 4.2 \times 10^{-4}$). Direct correlations between methylation levels and gene expressions were also observed ($p < 1 \times 10^{-3}$). Finally, associations with insulin resistance ($p < 3 \times 10^{-3}$) and steatosis ($p < 3 \times 10^{-5}$) were detected.

Conclusion: We identified the first epigenetic markers associated with T2D risk in the human liver tissue. DNA methylation information can be used to identify new pathways relevant to the pathophysiology of T2D and novel targets for disease prevention and treatment.

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Identification of DNA methylation and mRNA expression changes associated with age in human pancreatic islets

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Background and aims: Increased age is associated with a reduced capacity for insulin secretion and an increased risk of type 2 diabetes. Recent studies suggest that epigenetic modifications, such as DNA methylation, play a role in the development of type 2 diabetes. DNA methylation patterns has been shown to change significantly with age in tissues such as blood and muscle, but if this is the case also in human pancreatic islets has not yet been studied.

Materials and methods: Microarrays were used to analyse mRNA expression and DNA methylation in human islets from 87 non-diabetic donors with an age spanning between 26–74 years. A linear regression analysis was used to determine which methylation sites and probesets associate significantly with age.

Results: DNA methylation data of 482,889 sites remained after quality control and the methylation level of 322 of these were significantly associated with age after correction for multiple testing. These methylation sites correspond to 214 individual genes, including loci previously associated with diabetes. Interestingly, all of the significant methylation sites showed increased DNA methylation with increasing age. Moreover, based on the annotation of the analyzed methylation sites, we found enrichment of significant sites on chromosomes 2 and 19 and in the following gene regions; TSS1500 (the region 1500 bases upstream of the transcription start site), TSS200 and the 1st exon as well as in CpG islands. Seven genes exhibited significant associations for both DNA methylation and mRNA expression with increased age. Of these, 5 showed an inverse relation, with increased methylation and reduced expression. These 5 genes encode for a ribosomal protein, a putative

zinc finger transcription factor, a histone, a mitochondrial protein and a RNA methyl transferase protein, respectively.

Conclusion: Together, our study demonstrates that ageing is associated with differential DNA methylation in human pancreatic islets. These epigenetic modifications may contribute to altered gene expression, impaired insulin secretion and finally affect the risk for type 2 diabetes.

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Spermatozoa from lean and obese human carry distinct epigenetic signatures

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Background and aims: Obesity is associated with metabolic dysfunction and higher prevalence of related diseases such as Type 2 Diabetes. In humans, epidemiological evidence exists showing that paternal obesity can predispose offspring to obesity, regardless of the body mass index of the mother. Moreover, recent animal studies have shown that paternal nutritional status at time of mating can affect the metabolic health of the offspring, suggesting that spermatozoa carry environmental effects to the next generation. Here, we hypothesize that spermatozoa from obese human present a specific epigenetic content that may predispose to metabolic dysfunction.

Materials and methods: Using deep-sequencing, we comprehensively profiled small RNA content, DNA methylation and the histone retention profile of ultra pure motile spermatozoa fractions collected from 11 obese (BMI > 30), insulin resistant, and 16 lean (BMI 20–25), normal glucose tolerant men in their reproductive age (20–40 y).

Results: Analysis revealed a subset of RNAs being differentially expressed in obesity, including known as well as new small RNA species. Notably, piRNA-associated genes were found to control epigenetic process and metabolic functions. We found regions differentially methylated in obesity. Interestingly, around 25% were located in putative regulatory sites, and at close proximity of genes involved in development and the regulation of metabolic processes. Positioning of histones, on the contrary, was not affected by obesity.

Conclusion: Here we show that obesity is associated with an altered epigenetic signature in spermatozoa. The identified differentially expressed RNA molecules and differentially methylated regions have the potential to alter gene expression in the developing embryo and participate in the predisposition of metabolic dysfunction and related comorbidities.

Clinical Trial Registration Number: H-1-2011-077

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Variation in phenotype and DNA methylation is detectable in an observational cohort of young adult Bangladeshis exposed to famine during in utero and postnatal life

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Background and aims: Periconceptual, in utero and postnatal exposure to nutritional deficits may 'programme' cardiometabolic disease in later life. Recent human studies suggest that epigenetic variation may be associated with periconceptual nutritional deficiency, but this has not been described with postnatal exposures or replicated across studies. Aim: 1. To identify whether Bangladeshi adults exposed to famine in utero and/or in postnatal life have a programmed phenotype 2. To detect DNA methylation variation in these offspring on a genome-wide scale and at previously described targets.

Materials and methods: A cross-sectional cohort of adults (27–32 years) exposed to severe famine during 1974–5 in Matlab, Bangladesh was studied in three groups: 1. in utero exposed, 2. postnatal exposed, 3. Unexposed.

Approach: • Clinical phenotype (n=219) • Whole blood DNA bisulphite conversion (n=159) for (i) epigenome profiling via Illumina 450k Methylation

array, (ii) targeted pyrosequencing of published methylation variants: IGF2, PAX8, ZFYVE28, EXD3, BOLA3 • Validation using an external dataset

Results: The in utero exposed group had an excess of underweight individuals (49%) compared to postnatal exposed (32%) or unexposed (30%); more postnatal exposed offspring (26%) were overweight compared to in utero exposed (13%) or unexposed (14%) (proportional odds model $df=2$, deviance ratio 3.26, $p=0.039$). Of the underweight, in utero exposed had a higher mean glucose 120minutes post-glucose challenge (5.8mmol/l) compared to postnatal exposed or unexposed (both 4.8mmol/l) (ANOVA $p<0.02$). The Illumina 450k array identified differential methylation between the 3 groups at 416 probes (ANOVA $p\leq 0.001$) but these did not withstand correction for false discovery. Sixty-two regions showed methylation differences of $\geq 5\%$; most (65%) were from the comparison of postnatal exposure to unexposed. KEGG pathway analysis suggested potential impact on pathways involved in insulin signalling, axon guidance and ABC transporters. We found a 3-fold enrichment of published epigenomic variants in human islets in type 2 diabetes in our dataset ($p=0.01$). Bisulphite-pyrosequencing and/or array data showed small ($<7\%$) methylation differences at PAX8 and ZFYVE28, consistent with metastable epialleles in Gambian children exposed to variable periconceptual nutrition. Small methylation differences ($<4\%$) at IGF2 were consistent with the published Dutch Winter Hunger studies but are of uncertain significance.

Conclusion: Our data identifies phenotypic evidence of developmental programming of adult cardiometabolic disease from both in utero and postnatal famine exposure. We identify epigenetic differences in DNA methylation across multiple genomic regions, but larger studies are needed to control for false discovery. Via pathway analysis and comparison to external datasets, we suggest possible functional roles in insulin signaling and via pancreatic islet function. We replicate small quantitative differences in DNA methylation at targets previously associated with famine exposure. Future studies need larger sample sizes, longitudinal sampling and intervention to determine causality and potential reversibility.

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OP 42 Liver metabolism

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New biomarkers of NAFLD: results from plasma metabolomic analysis

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Background and aims: Non Alcoholic Fatty Liver Disease (NAFLD) is associated with both peripheral and hepatic insulin resistance and is an important risk factor for both diabetes and cardiovascular disease. Metabolomic techniques aim to identify new biomarkers related to alteration in organ metabolism. We have performed metabolomic analysis of plasma samples of subjects with NAFLD to find possible markers of metabolic dysfunction and liver disease.

Materials and methods: We studied 54 subjects, 45 NAFLD with liver biopsy and 9 healthy control (CT) and measured plasma concentration profile of amino acids (AA) and fatty acid (FA) by GCMS. Data were correlated with indexes of insulin resistance (IR) measured by tracers, LFTs, NAS and fibrosis score, beta-hydroxybutyrate (BOH), ox-LDL, total antioxidative status (TAS), sRAGE, and adiponectin. We calculated: a) using tracer infusion, hepatic IR as endogenous glucose production \times fasting insulin (H-IR=EGPxFPI), adipose tissue insulin resistance (AT-IR) as fasting lipolysis \times FPI; b) from fatty acid composition we calculated de novo lipogenesis index (DNL=16:0/18:2) and SCD1 activity as 16:1/16:0 from AA profile, the branched chain concentrations (BCAA) and the ratio of glutamate/(glycine +serine), that is as an index of glutathione biosynthesis (GSH-I).

Results: Subjects with NAFLD had increased H-IR (118 ± 10 vs. 56 ± 7) and AT-IR (34 ± 3 vs. 13 ± 2), GSH-I (0.73 ± 0.06 vs. 0.33 ± 0.09), DNL (1.17 ± 0.05 vs. 0.86 ± 0.04) and SCD1 (0.10 ± 0.01 vs. 0.08 ± 0.01) indices compared to controls. GSH-I, DNL and SCD1 were increased in proportion to the degree AT-IR ($r=0.28$; $r=0.41$; $r=0.43$) and also with the increase of H-IR ($r=0.39$; $r=0.28$; $r=0.32$). GSH-I was increased proportionally to the severity of fibrosis ($r=0.36$ per trend, $p<0.0001$), ln(cholesterol) ($r=0.39$) and negatively with ln(sRAGE) ($r=0.30$) but not with TG liver content nor with NAS score. TAS, ox-LDL and adiponectin. DNL was increased proportionally to circulating ln(TG) ($r=0.53$), fat in biopsy ($r=0.45$) and fibrosis ($r=0.52$).

Conclusion: Metabolomic analysis allowed the identification of indices (GSH-I, DNL and SCD1) associated with increased hepatic and adipose tissue insulin resistance and severity of fatty liver disease.

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Orphan nuclear receptor Nur77 mediates fasting-induced hepatic FGF21 expression

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Background and aims: The fasting-induced hepatic hormone, fibroblast growth factor 21 (FGF21), is an intriguing candidate for the treatment of metabolic syndromes. Although PPAR α is known to play a major role in induction of hepatic FGF21 expression, other fasting-induced transcription factors in FGF21 expression has not been fully studied. In the present study, we investigated whether the fasting-induced activation of the transcription factors Nur77 increases hepatic FGF21.

Materials and methods: We examined the effects of glucagon/cAMP, a well-known hormone induced by fasting, on hepatic Nur77 and FGF21 expression in vivo and in vitro. To elucidate the association between Nur77 and FGF21 expression, we examined whether adenovirus-mediated over-expression of Nur77 (Ad-Nur77) increases FGF21 production and knockdown of Nur77 with siRNA abolished forskolin-induced FGF21 expression. To further con-

firm that Nur77 transcriptionally activates FGF21 expression, ChIP assay, EMSA and mutagenesis analysis were employed.

Results: We found that fasting induced hepatic Nur77 and FGF21 expression. Glucagon and forskolin increased Nur77 and FGF21 expression in vivo and in vitro, respectively. Ad-Nur77 increased FGF21 production in cultured hepatocytes. Hepatic overexpression of Nur77 via tail vein injection of Ad-Nur77 increased FGF21 mRNA and protein levels. Moreover, siRNA-Nur77 abolished the effect of forskolin on FGF21 expression. The results from ChIP assay, EMSA and mutagenesis analysis showed that Nur77 bound to putative NBRE of FGF21 promoter in cultured hepatocytes and fasting induced Nur77 binding to FGF21 promoter in vivo.

Conclusion: This study shows that Nur77 mediates fasting-induced hepatic FGF21 production. The present study suggests an alternative mechanism by which hepatic FGF21 transcription is mediated under fasting conditions.

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Overexpression JAZF1 protected ApoE^{-/-} mice from atherosclerosis by inhibiting hepatic cholesterol synthesis

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Background and aims: Genome wide association studies have suggested an association of Juxtaposed with another zinc finger gene 1 (JAZF1) with type2 diabetes mellitus (T2DM). As an inhibitor of the TAK1/TR4 signaling pathway, JAZF1 has been shown to be involved in gluconeogenesis, lipid metabolism and insulin sensitivity. However, its role in insulin resistance and atherosclerosis in vivo remains unknown. The present study was designed to investigate in vivo the impact of JAZF1 on insulin resistance-associated dyslipidemia and atherosclerosis.

Materials and methods: We established Adenovirus-mediated JAZF1 overexpression to characterize the role of JAZF1 in the regulation of lipid metabolism and the development of atherosclerosis in normal chow- or high fat diet (HFD)-fed ApoE^{-/-} mice. We use euglycaemic-hyperinsulinaemic clamping to examine Insulin sensitivity, Cholesterol *de novo* synthesis was measured by intraperitoneal acetate injection and atherosclerotic plaques were quantified by histological analysis. A dual-luciferase reporter assay was used to assess the ability of JAZF1 to regulate HMGCR transcriptional activity, mRNA and protein expressions were measured by qRT-PCR and Western blot, respectively.

Results: we showed that JAZF1 overexpression improved HFD-induced hepatic insulin resistance in C57BL/6J mice. In HFD-fed ApoE^{-/-} mice, JAZF1 overexpression decreased serum cholesterol levels and hepatic cholesterol synthesis by inhibiting CREB dependent 3hydroxy3methylglutaryl coenzyme A reductase (HMGCR) promoter transcriptional activity and JAZF1 overexpression had significantly reduced aortic and aortic sinus en face and cross-sectional plaque areas in HFD-fed ApoE^{-/-} mice.

Conclusion: we report the first evidence that JAZF1 overexpression protected against development of atherosclerosis and insulin resistance induced by a HFD in apoE^{-/-} mice and in C57BL/6J mice. Furthermore, we provide molecular explanation by which JAZF1 can regulate hepatic cholesterol synthesis and HMGCR transcriptional activity via CREB-dependent mechanisms. Our data delineate that JAZF1 overexpression may represent a promising strategy for the treatment of insulin resistance-associated dyslipidemia and atherosclerosis.

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Defective autophagy results in reduced glycogen breakdown in the liver of IUGR newborn Wistar rats. Consequences for glucose homeostasis

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Background and aims: Glycogen autophagy is a highly regulated process that represents a mechanism of glucose homeostasis under conditions of demand for the production of this sugar as in the liver of newborn animals. The abrupt interruption of the maternal glucose supply with birth, leads to the development of hypoglycaemia in the newborn. This promotes glucagon secretion and triggers the normal mechanisms for the newborn to adapt to the postnatal environment. Glucagon acts through the cAMP/protein Kinase A pathways and induces glycogen autophagy. We previously showed that intrauterine growth restriction (IUGR) induced massive glycogen accumulation in the liver of fetuses and neonates. Interestingly, IUGR neonates were slightly hypoglycaemic and showed hypoglucagonaemia and glucagon resistance but insulin hypersensitivity. In this study we set out to examine the autophagic response in the liver of IUGR newborns and whether this process might be linked to the development of insulin resistance associated to IUGR. **Materials and methods:** Newborn rats naturally delivered from pregnant females fed ad libitum or 65% food-restricted from the last third of gestation, were kept separated from their mothers and used 3 or 6h after birth. Liver glycogen content was quantified by a KOH-ethanol precipitation and acid hydrolysis method. The protein levels of the enzymes involved in glucose metabolism, the markers of autophagy and ER stress were determined by western blot in hepatic homogenizes. Ultrastructural analysis of the neonatal liver was performed by electron microscopy.

Results: At birth (0h), liver glycogen values of control (C) and IUGR rats were 23.8 ± 3.2 and 57.4 ± 7.3 mg/g ($p < 0.01$), respectively. In C rats, the amount of glycogen precipitously diminished at 3 and 6h of age whereas glycogen stores remained significantly elevated in restricted newborns. In agreement, glycogen synthase was found more active in IUGR group at both 3 and 6h after birth, the phosphorylated form of GSK3 increased and glycogen phosphorylase and PEPCK protein levels diminished ($p < 0.01$). To address the implication of autophagy in the lack of glycogen mobilization in restricted newborns we measured autophagy markers at 3 and 6h after birth. C newborns fasted 6h, showed a marked induction of LC3B II conversion. In contrast, IUGR newborns displayed defective autophagy at this time. Electron microscopic examination showed enhanced autophagic activity and consumption of liver glycogen stores from 3 to 6h after birth in C newborns whereas in IUGR rats the formation of glycogen-containing vacuoles were rare and vast areas of cytoplasmic glycogen were observed at both 3 and 6h of age. Alpha glucosidase enzyme levels increased at 6h of postnatal life in both groups of animals. When fed a high-fat diet during 22 weeks, C rats showed enhanced autophagic activity in liver however, IUGR rats maintained lower levels of Atg7 and LC3B II and developed insulin resistance, liver steatosis and ER stress.

Conclusion: We suggest that defective autophagy may lead to ineffective macromolecule turnover, such as glycogen or lipids in IUGR individuals and consequently compromise hepatic metabolic function favouring insulin resistance development.

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OP 43 GLP-1 analogues: novel formulations

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Benefits of a fixed-ratio formulation of once-daily insulin glargine/lixisenatide (LixiLan) vs glargine in type 2 diabetes inadequately controlled on metformin

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Background and aims: LixiLan is a fixed-ratio formulation combining insulin glargine (GLARG) with lixisenatide (Lixi) in a single pen device currently in development for the management of type 2 diabetes mellitus (T2DM). This randomized, open-label study assessed the efficacy and safety of LixiLan (GLARG 2 U / Lixi 1 µg) vs GLARG in insulin-naïve T2DM on top of metformin.

Materials and methods: Patients (mean baseline A_{1c} 8.0%, BMI 32.1 kg/m², diabetes duration 6.7 yrs) were randomized to LixiLan (n=161) or GLARG (n=162) for 24 weeks. Primary objective was non-inferiority in A_{1c} reduction; if non-inferiority met, statistical superiority was tested.

Results: At 24 weeks, mean A_{1c} was reduced to 6.3% and 6.5% with LixiLan and GLARG respectively, establishing statistical superiority of LixiLan (LS mean difference: -0.17% [-0.312% to -0.037%; p=0.0130]), and 84% and 78% achieved A_{1c} <7%, respectively (Table). Despite substantial reductions of A_{1c}, body weight was reduced with LixiLan (p<0.0001), with no increase in documented (≤70 mg/dL [3.9 mmol/L]) hypoglycaemic events (22% and 23%, respectively) and no severe hypoglycaemia. Composite endpoints of A_{1c} <7% with no weight gain, or no weight gain and no hypoglycaemia were achieved significantly more often with LixiLan. Incidence of nausea and vomiting was only 7.5% and 2.5%, respectively, with LixiLan.

Conclusion: LixiLan achieved robust A_{1c} reductions to 6.3% with weight loss and no increased hypoglycaemia vs GLARG, with very low gastrointestinal adverse events in T2DM inadequately controlled on metformin.

Efficacy parameters in mITT population of NCT01476475		LixiLan Glargine/Lixisenatide fixed-ratio final dose* LS Mean 36 U / 18 µg once daily at Week 24 (LOCF) (n=161)	Glargine alone final dose* LS Mean 39 U once daily at Week 24 (LOCF) (n=162)
A _{1c} (%)	Mean baseline ± SD Mean Week 24 ± SD (LOCF) LS mean ± SE change from baseline to Week 24 (LOCF) LS mean difference vs glargine [95% CI; p-value vs glargine]	8.06 ± 0.79 6.31 ± 0.72 -1.82 ± 0.058 -0.17 [-0.312 to -0.037; p=0.0130]	8.01 ± 0.81 6.47 ± 0.64 -1.64 ± 0.057
Proportion achieving A _{1c} <7% (%)	n (%) Week 24 (LOCF) % difference vs glargine [95% CI]**	135 (84.4) 6.2 [-2.16 to 14.47]	126 (78.3)
Body weight (kg)	Mean baseline ± SD Mean Week 24 ± SD (LOCF) LS mean ± SE change from baseline to Week 24 (LOCF) LS mean difference vs glargine [95% CI; p-value vs glargine]	90.26 ± 17.63 89.10 ± 16.89 -0.97 ± 0.289 -1.44 [-2.110 to -0.773; p<0.0001]	91.70 ± 16.62 92.09 ± 16.30 0.48 ± 0.282
2-h PPG (mg/dL)***	Mean baseline ± SD Mean Week 24 ± SD (LOCF) LS mean ± SE change from baseline to Week 24 (LOCF) LS mean difference vs glargine [95% CI; p-value vs glargine]	289.61 ± 65.27 153.33 ± 58.22 -134.99 ± 5.090 -57.07 [-69.028 to -45.113; p<0.0001]	279.32 ± 69.84 208.01 ± 50.97 -77.92 ± 4.930
A _{1c} <7% with no weight gain	n (%) Week 24 % difference vs glargine [95% CI]	90 (56.3) 19 (8.57 to 29.51)	60 (37.3)
A _{1c} <7%, no weight gain and no documented symptomatic hypoglycaemia****	n (%) Week 24 % difference vs glargine [95% CI]	74 (46.3) 17.7 [7.46 to 27.97]	46 (28.6)

*On top of metformin; **Weighted average of proportion difference between treatment arms from each randomization strata (A_{1c} [≤8.0%, ≥8.0%] and BMI [≤30 or ≥30 kg/m²]) using Cochran-Mantel-Haenszel weights; ***After a standardized meal test; ****Plasma glucose concentration ≤70 mg/dL [3.9 mmol/L]; CI=confidence interval; LixiLan=Glargine/Lixisenatide; LOCF=last observation carried forward; LS=least squares; mITT=modified intent-to-treat; PPG=postprandial glucose; SD=standard deviation; SE=standard error

Clinical Trial Registration Number: NCT01476475

Supported by: Sanofi

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Efficacy and tolerability of ITCA 650 (continuous subcutaneous exenatide) in poorly controlled type 2 diabetes with baseline HbA_{1c} >10%

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Background and aims: ITCA 650, the injection-free GLP-1 receptor agonist that provides continuous SC exenatide for up to 12 months from a single subdermal placement, is undergoing extensive clinical evaluation in multiple Phase 3 double-blind studies. This report represents the first 6 month, open-label experience with ITCA 650 mini-pumps from an ongoing multicenter study in subjects with type 2 diabetes who did not meet enrolment criteria for the double-blind placebo controlled trial because of A1C >10%.

Materials and methods: Entrance criteria for this open-label trial were: A1C >10% to ≤12%, age 18-80 years, BMI 25-45 kg/m², and on stable (≥3 months) diet and exercise and/or monotherapy or any combination of metformin, sulfonylurea, and thiazolidinedione. Treatment was initiated by placing a 3-month ITCA 650 mini-pump delivering 20 mcg/day, which was then replaced by a 6-month ITCA 650 mini-pump delivering 60 mcg/day for 26 weeks. Pre-study oral antidiabetic agents (OADs) were maintained unchanged for the 39 week of treatment. The primary endpoint was change in A1C from baseline to week 39.

Results: At the time of this initial interim analysis, 50, 39, and 25 of the 60 subjects enrolled had completed 13, 19, and 26 weeks of treatment; respectively. Mean baseline characteristics for the entire cohort (n=60) were A1C 10.7%, age 52.1 yrs, BMI 32.1 kg/m², duration of diabetes 8.9 yrs, OAD use 69%. Mean reductions of A1C at Weeks 13 (n=50), 19 (n=39), and 26 (n=25) were -2.5%, -2.9%, and -3.2%, respectively. A1C reductions ≥2% were achieved by 78% of subjects who completed at least 13 weeks of treatment; 50% achieved >3% and 22% achieved ≥4% reductions. A1C targets of <7% were achieved in 22% of subjects who had completed at least 13 weeks of treatment. Adverse events were consistent with previous trials with ITCA 650.

Conclusion: ITCA 650 has the potential to markedly improve glycemic control in patients with severe hyperglycaemia and longstanding diabetes.

Clinical Trial Registration Number: NCT01785771

Supported by: Intarcia Therapeutics

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IDegLira, a combination of insulin degludec and liraglutide, improves both pre- and postprandial plasma glucose in patients with type 2 diabetes

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Background and aims: Pre- and postprandial glucose levels contribute importantly to overall glycaemic control and consequently clinical outcomes. This analysis explored whether a novel combination of insulin degludec (IDeg) and liraglutide (Lira), IDegLira, enabled more patients to reach recommended pre- and postprandial target ranges, compared with administration of its individual components.

Materials and methods: In this *post hoc* analysis of two phase 3 trials: DUAL I (IDegLira vs. IDeg and Lira in patients uncontrolled on oral antidiabetic drugs [OADs]; 52 weeks) and DUAL II (IDegLira vs. IDeg in patients uncontrolled on basal insulin + OADs; 26 weeks), we investigated at end of trial (EOT) the proportion of patients with self-measured blood glucose (BG) values within the pre-prandial target range of ≥3.9 to ≤7.2 mmol/L and postprandial target of <9 mmol/L with IDegLira compared with IDeg or Lira alone. Postprandial BG values were assessed 90 minutes after breakfast, lunch and dinner. Preprandial BG was measured before breakfast, lunch, dinner and breakfast the following day. In addition, the full 9-point profile included measurements before bedtime and at 4am. Finally, we compared the proportion of patients whose entire 9-point BG profile was between ≥3.9 and <9 mmol/L with IDegLira and IDeg or Lira alone.

Results: At baseline, the proportion of patients with BG values within the target ranges was similar across treatments in the two trials. At EOT, the proportion of subjects with all three postprandial BG values <9 mmol/L was significantly higher for patients treated with IDegLira (DUAL I 51%; DUAL II 37%) than those treated with IDeg (DUAL I 38%; DUAL II 25%) or Lira

(36%). The likelihood of achieving all four pre-prandial BG values within the recommended range (≥ 3.9 to ≤ 7.2 mmol/L) was significantly greater with IDegLira (DUAL I 48%; DUAL II 44%) compared with IDeg (DUAL I 41%; DUAL II 27%) or Lira (32%). At EOT, the proportion of patients with all 9 BG values within the range ≥ 3.9 to < 9 mmol/L was significantly higher for patients treated with IDegLira (DUAL I 39%; DUAL II 32%) than for patients treated with IDeg (DUAL I 28%; DUAL II 20%) or Lira (31%).

Conclusion: Treatment with IDegLira resulted in a greater likelihood of patients having both pre- and postprandial BG values within target ranges compared with either IDeg or Lira. Improved pre- and postprandial BG levels suggest that the predictability of glycaemic control within one day is increased with IDegLira.

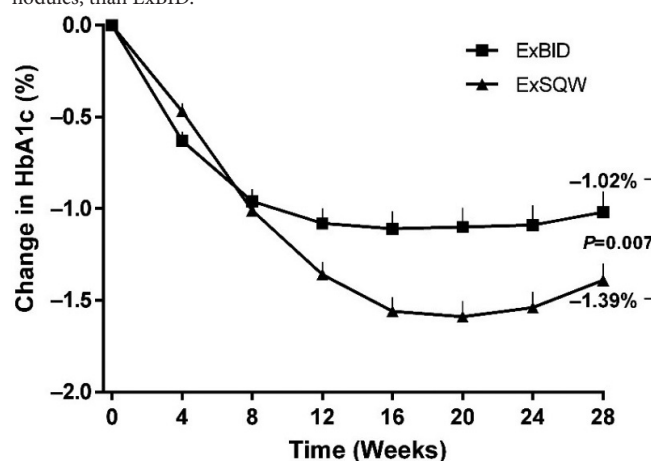
Key results	DUAL I			DUAL II		
	IDegLira n=833	IDeg n=413	Lira n=414	IDegLira vs. IDeg at EOT, OR [95% CI]	IDegLira vs. Lira at EOT, OR [95% CI]	IDegLira vs. IDeg at EOT, OR [95% CI]
Postprandial BG (3 meals) < 9 mmol/L						
N	799	399	399	1.77	1.93	1.90
Baseline: all 3 BG values < 9 mmol/L, %	7	4	7	[1.37; 2.28]	[1.49; 2.49]	[1.17; 3.07]
EOT: all 3 BG values < 9 mmol/L, %	51	38	36	($p < 0.0001$)	($p < 0.0001$)	($p = 0.0093$)
Pre-prandial between 3.9–7.2 mmol/L						
N	792	403	396	1.34	2.06	2.29
Baseline: all 4 BG values within target, %	3	3	6	[1.05; 1.72]	[1.59; 2.67]	[1.41; 3.73]
EOT: all 4 BG values within target, %	48	41	32	($p = 0.0204$)	($p < 0.0001$)	($p = 0.0008$)
All values ≥ 3.9 and < 9 mmol/L						
N	746	374	366	1.79	1.46	2.07
Baseline: all 9 BG values within target, %	4	2	6	[1.36; 2.36]	[1.12; 1.92]	[1.22; 3.49]
EOT: all 9 BG values within target, %	39	28	31	($p < 0.0001$)	($p = 0.0059$)	($p = 0.0067$)

BG, blood glucose; CI, confidence interval; EOT, end of trial; IDeg, insulin degludec; Lira, liraglutide; LOCF, last observation carried forward; OR, odds ratio

Clinical Trial Registration Number: NCT01336023, NCT01392573

Supported by: Novo Nordisk

Conclusion: ExSQW with autoinjector delivery simplified treatment with robust glycaemic control, weight loss and less GI AEs, but more injection-site nodules, than ExBID.



Clinical Trial Registration Number: NCT01652716

Supported by: Bristol-Myers Squibb/AstraZeneca

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DURATION-NEO-1: greater HbA_{1c} reductions with exenatide suspension once weekly by autoinjector pen vs exenatide twice daily in inadequately controlled type 2 diabetes

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Background and aims: Once-weekly (QW) exenatide consistently improves glycaemic control and promotes weight loss in patients with type 2 diabetes mellitus (T2DM) but requires reconstitution and may be difficult to use for some patients. A QW suspension containing exenatide dispersed in microspheres suspended in a triglyceride carrier, was developed to eliminate the need for reconstitution and allow delivery via a single-use autoinjector pen. This simplified approach may enhance adherence. An open-label, 28-week study compared exenatide suspension QW (ExSQW) to exenatide twice daily (ExBID) in patients with inadequately controlled T2DM.

Materials and methods: Patients treated with diet/exercise or combination of oral medications (metformin, sulfonylurea or pioglitazone) were randomized (in a 3:2 ratio) to ExSQW 2 mg (n=229) or ExBID 10 mcg (n=148). The primary endpoint was change in HbA_{1c} from baseline to Week 28 with ExSQW vs ExBID.

Results: Baseline characteristics were similar in both groups: mean±SD HbA_{1c} of 8.48±1.03%, fasting plasma glucose (FPG) of 182±45 mg/dL (corresponding to 10.10±2.53 mmol/L), and body weight of 96.9±21.0 kg. Changes in HbA_{1c} from baseline are shown in Figure 1; at Week 28, the reduction (least-squares [LS] mean±SE) in the ExSQW group was greater than in the ExBID group (-1.39±0.09 vs -1.02±0.11%; P=0.007). Achievement of HbA_{1c} <7% was comparable in the ExSQW vs ExBID groups (49% vs 43%; P=0.225). Similar FPG reductions were observed in both groups as early as Week 2. By Week 28, FPG reductions reached -32.7±3.9 and -22.5±4.9 mg/dL (corresponding to -1.81±0.22 and -1.25±0.27 mmol/L) for ExSQW and ExBID, respectively (P=0.166). Body weight decreased similarly in the ExSQW (-1.49±0.28 kg) and ExBID (-1.89±0.36 kg) groups. Gastrointestinal (GI) adverse events (AEs) were less frequent in the ExSQW group than in the ExBID group (nausea: 9.6% vs 21.2%; diarrhea: 5.2% vs 11.6%; vomiting: 3.5% vs 6.2%, respectively), but injection-site nodules occurred more often (12.7% vs 0.7%). No major hypoglycaemia was reported; minor hypoglycaemia was infrequent, generally mild, and occurred primarily in patients with concomitant sulfonylurea use. The ExSQW and ExBID groups, respectively, had low rates of serious AEs (2.6% and 4.8%) and withdrawals due to AEs (2.2% and 4.8%). The completion rate was slightly greater with ExSQW (86.0% vs 79.7%).

OP 44 Diabetes and cancer

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Cancer occurrence in type 1 diabetes patients: a 4-country study with 8800 cancer cases in 3.7 mio person-years

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Background and aims: Diabetes (DM) patients carry an excess risk of cancer in the order of 20–25%; this is mainly derived from follow-up of type 2 patients (T2D). The excess risk of cancer among type 1 (T1D) patients is described here as it is anticipated to be different from T2D patients.

Materials and methods: T1D patients from four countries with nation-wide diabetes registers: Australia (1997–2008), Denmark (1995–2009), Finland (1972–2010), and Sweden (1987–2011) were followed for cancer occurrence. T1D was defined by diagnosis of DM before age 30. Cancer incidence rates were compared to population cancer incidence rates from national cancer registries. We used Poisson-models for rates, adjusting for age and date of follow-up, and date of birth. We estimated the overall rate ratio (RR) for all T1D patients and the effect of time since DM diagnosis.

Results: There were a total of 8,807 cancers among T1D patients during 3.7 million person-years of follow-up with median age at cancer diagnosis 51.1 (IQR: 43.5–59.5). Overall, we found an RR of any type of cancer of 1.00 (95% CI: 0.97–1.03) among men and 1.05 (95% CI: 1.02–1.08) among women. The highest RRs were found for colorectal cancer, (RR=1.13 (M), 1.14 (F)), liver cancer (RR=2.14 (M), 1.50 (F)), pancreas cancer (RR=1.74 (M), 1.31 (F)), endometrial cancer (RR=1.4 kidney cancer (RR=1.28 (M), 1.44 (F)), and thyroid cancer (RR=1.29 (M), 1.46 (F)), all significant. We found a strong effect of diabetes duration, with an RR of 2.5 during the first year, decreasing to 1.2 (M) and 1.1 (F), after 2–5 years.

Conclusion: Some of the observed excess risk may be explained by risk factors for cancer being more frequent in T1D patients (obesity), however this effect is presumably smaller than in T2D patients, and hence consistent with the smaller excess risk. The long-term RR (>5 years of DM) is less than 1.2, which means that some small effect of exogenous insulin cannot be excluded, but the study is also consistent with an assumption of no such effect.

Supported by: EFSD

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Time-varying incidence of cancer after incident type 2 diabetes: differences by obesity-versus non-obesity-related cancers

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Background and aims: People with Type 2 diabetes (T2D) are at increased incident risk of several cancer types, many of which are also causally linked with excess body weight. To explore these relationships further, we determined the incidence of cancer in people with incident T2D during different time windows following diabetes diagnosis, stratified as obesity- and non-obesity-related cancers.

Materials and methods: We used the Salford Integrated record database linked with the Northwest cancer Intelligence service (UK) for data from 1995–2010. We identified incident cohorts with (N = 10,328) and without T2D, who were matched (1:2) by age, sex and index year. Following exclusion of previous cancers, first site-specific cancers were identified prospectively in both cohorts. Obesity-related cancers were post-menopausal breast, colorectal, endometrial, kidney, gallbladder, pancreas, liver, and ovarian. Risk estimates were expressed as hazard ratios (HRs) and their 95% confidence intervals (CIs).

Results: Diabetic patients had a significantly higher BMI than matched controls (mean 30.9 (SD6.7) vs 27.3 (SD5.4)), and were more likely to be ever smokers (60.0% vs 52.63%). Within 6 months following diabetes onset, participants with T2D were at increased risk of obesity-related (HR 1.54, 95% CI: 0.98, 2.40) but not non-obesity-related cancers. From 6 months to 10 years,

risk associations for obesity- and non-obesity-related cancers remained close to null. After 10 years following diabetes onset, participants with T2D were at increased risk of non-obesity-related (HR 1.49, 95% CI: 1.13, 1.97) but not obesity-related cancers. These observations were robust to adjustment for smoking.

Conclusion: We confirmed that people with incident T2D are at increased risk of certain cancers; the risk for obesity-related cancers is particularly elevated near to the time of diabetes onset, and is likely to present detection time bias. Paradoxically, we noted elevated risk of non-obesity-related cancers among people with T2D with greater than 10 years disease duration, emphasizing the importance of assessing diabetes-cancer associations using time-varying approaches.

Supported by: EFSD

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Diabetes and cancer risk in a population-based study with 20 years of follow-up: the Rotterdam Study

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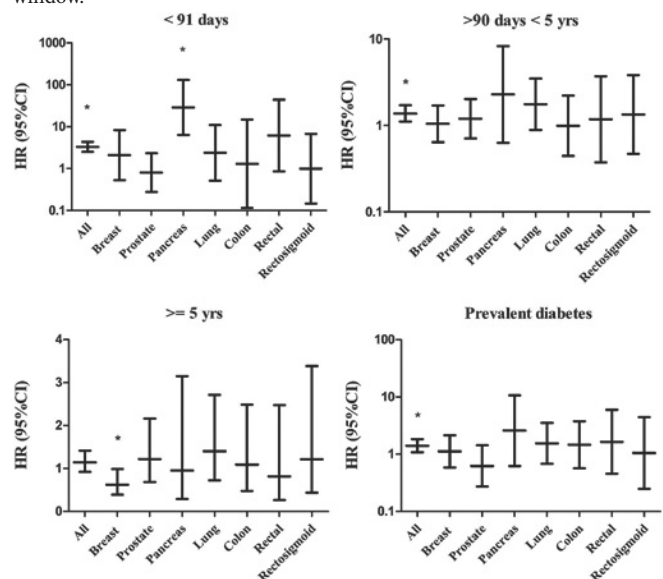
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Background and aims: Type 2 diabetes is associated with an increased risk of cancer. Most observational studies on this topic analyze diabetes as a dichotomous risk factor without adjusting for diabetes Duration before cancer occurrence. The objective of this study was to investigate the association between diabetes duration and cancer risk in more detail.

Materials and methods: In this prospective cohort study, incident diabetes was diagnosed on the basis of clinical information and use of glucose-lowering medication. Details on incident cancers were obtained via general practitioners and linkage to registers for pathology. Time-dependent Cox proportional hazard models were used. Additionally, a five year latency period was taken into account.

Results: The study comprised 10,746 individuals. Diabetes was associated with an increased overall risk of incident cancers (HR 1.22, 95%CI 1.07–1.39). Distinguished by type of cancer, diabetes was associated with an increased risk of breast (HR 1.50, 95%CI 1.05–2.15) and pancreatic cancer (HR 2.93, 95% CI 1.75–4.89), but with a decreased risk of prostate cancer (HR 0.59, 95%CI 0.38–0.93). As a diabetes diagnosis of less than three months before a cancer diagnosis was associated with strongly increased risks for all cancers (HR 3.30, 95%CI 2.50–4.32) and pancreatic cancer (HR 28.74, 95%CI 6.32–130.58), at least a part of the association might be explained by detection bias or by protopathic bias. After adjusting for a latency period of five years, the association regarding the decreased risk of prostate cancer remained statistically significant, whereas the risk of rectal cancer increased (HR 2.26, 95%CI 1.04–4.95).

Conclusion: Although the magnitude of the association between diabetes and increased risk of cancer seems to be inflated by detection- or protopathic bias, an independent association remains. Future studies investigating this association should adjust for diabetes duration and a plausible etiological risk window.



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Cancer therapy alters whole body metabolism later in life: a potential role for epigenetic mechanismsV. Nylander¹, D. Simar², M. Aznar³, L. Specht³, J.R. Zierath¹, R. Barres¹;¹The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark, ²School of Medical Sciences, University of New South Wales, Sydney, Australia, ³University of Copenhagen, Denmark.

Background and aims: Cancer survivors are at increased risk of acquiring metabolic dysfunction such as glucose intolerance and Type 2 Diabetes, years after end of cancer treatment. Epidemiological studies identified total body irradiation as the highest risk factor. Given the importance of muscle tissue in whole body glucose metabolism, we focused on skeletal muscle progenitor cells. We hypothesized that irradiation causes epigenetic changes in this cell type that could account for the delayed, deleterious effects on metabolic function. Aim: To determine if irradiation alters the DNA methylome of muscle progenitor cells, leading to altered differentiation potential and metabolic dysfunction.

Materials and methods: Male C57Bl/6 mice were irradiated (IRR) or not (CTL) with one dose of total body irradiation (6 Gy). Mice were allowed to recover from acute irradiation effects for 5 weeks before randomisation into groups fed CHOW or high fat diet (HFD, 60 % energy intake from fat). Metabolic characterization of mice was done by MR scan, glucose tolerance test and indirect calorimetry. Muscle progenitor cells were isolated by FACS sorting to investigate for deficiencies in intracellular pathways controlling metabolism or cell differentiation. In vitro experiments were performed using L6 rat myoblasts, which were irradiated with one dose of 1, 3 or 6 Gy. Cells were allowed to recover from irradiation for 4 weeks, before differentiation was induced and insulin signalling and DNA methylation was investigated. Statistical analysis used was one-way and two-way ANOVA for MR and body weight, and repeated measures analysis for indirect calorimetry data and glucose tolerance tests.

Results: After recovery from irradiation, calorie intake, oxygen consumption and energy expenditure were similar in both groups. Prolonged HFD induced impaired glucose homeostasis in IRR-HFD, but not in IRR-CHOW. This was accompanied by increased fasting insulin levels in IRR-HFD (50%, $p=0.033$), whereas fasting insulin was decreased in IRR-CHOW (20%, $p=0.022$), as compared to respective controls. Muscle progenitor cells collected 17 weeks post irradiation and cultured in vitro exhibited decreased growth rate (30%, $p=0.03$) compared to cells collected from CTL animals. Muscle cells collected from IRR animals showed altered signalling pathways controlling cell division and differentiation. Irradiation of rat muscle cell line phenocopied the impaired activation of signalling and exhibited an altered differentiation and DNA methylation profile.

Conclusion: Our study confirms that irradiation induces a long-term alteration of glucose metabolism and suggests that irradiation stably alters the epigenome and the differentiation potential of muscle progenitor cells. Irradiation-induced epigenetic modification could be a mechanism by which cancer survivors develop metabolic complications later in life.

Supported by: EFSD

OP 45 Diabetic foot: mechanisms of wound healing

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Overexpression of cutaneous mitochondrial superoxide dismutase in recently diagnosed type 2 diabetic patientsD. Ziegler^{1,2}, A. Strom¹, S. Püttgen¹, J. Brüggemann¹, I. Ziegler¹, B. Ringel¹, M. Roden^{1,2}, German Diabetes Study Group;¹Institute for clinical Diabetology, German Diabetes Center at Heinrich Heine University, ²Department of Endocrinology and Diabetology, University Hospital, Düsseldorf, Germany.

Background and aims: Oxidative stress resulting from enhanced free-radical formation and immune-mediated processes has been implicated in the pathogenesis of diabetic neuropathy. Since manganese superoxide dismutase 2 (SOD2) is responsible for superoxide detoxification in mitochondria, we hypothesized that cutaneous SOD2 could be overexpressed in recently diagnosed diabetes.

Materials and methods: We assessed skin biopsies and nerve function in 71 participants of the German Diabetes Study (GDS) with recently diagnosed type 2 diabetes (age: 54.5 ± 0.9 [SEM] years; male: 67.6%; BMI: 32.2 ± 0.7 kg/m², diabetes duration: 1.0 ± 0.1 years; HbA1c: $6.5 \pm 0.1\%$) and 52 healthy control subjects (age: 55.2 ± 1.2 years; male: 53.8%, 25.4 ± 0.5 kg/m²). Intraepidermal nerve fibre density (IENFD) was assessed by immunohistochemistry in 3-mm punch biopsies from the distal leg. Subepidermal SOD2 area was determined by immunofluorescence using a polyclonal SOD2 antibody. CD31 immunohistochemistry was used to quantify subepidermal endothelial cell area. Peripheral nerve function was assessed by motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception thresholds (VPT), and thermal detection thresholds (TDT), while cardiac autonomic nerve function was assessed by heart rate variability (HRV) in the time and frequency domains. All comparisons were adjusted for sex, age, and BMI.

Results: IENFD was lower by 24% in diabetic subjects vs controls (6.9 ± 0.3 vs 9.1 ± 0.4 fibres/mm; $P < 0.0001$). Subepidermal SOD2 area was increased by 60% in the diabetic group vs controls (0.24 ± 0.02 vs 0.15 ± 0.02 ; $P < 0.0001$). Subepidermal endothelial cell area did not differ between the diabetes group and controls (2.03 ± 0.15 vs $1.89 \pm 0.13\%$), but was smaller by 28% in diabetic subjects with known diabetes duration > 1 year compared to those who had diabetes for ≤ 1 year (1.64 ± 0.16 vs $2.29 \pm 0.22\%$; $P = 0.047$). Subepidermal endothelial cell area declined ($r = -0.33$; $P = 0.02$), whereas SOD2 area augmented with increasing diabetes duration ($r = 0.26$; $P = 0.032$). In the diabetes group, multivariate linear regression analyses revealed that subepidermal endothelial cell area was associated with IENFD ($\beta = 0.28$; $P = 0.048$), whilst SOD2 area was related to the low-frequency/high-frequency (LF/HF) ratio as an indicator of sympathovagal balance ($\beta = -0.384$; $P = 0.001$).

Conclusion: Patients with recently diagnosed type 2 diabetes show cutaneous SOD2 overexpression indicating enhanced local oxidative stress linked to cardiac sympathetic predominance as well as dermal microvascular alterations related to epidermal nerve fibre loss and diabetes duration. Whether cutaneous SOD2 is an early marker to predict the development of diabetic peripheral and autonomic neuropathy remains to be determined in prospective studies.

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Wound healing rate is impaired by a glucose-induced overactive Delta4-Notch1 loopX. Zheng¹, V.G. Sunkari¹, I.R. Botusan^{1,2}, J. Grünler¹, A.I. Catrina³, F. Radtke⁴, K. Brismar¹, S.-B. Catrina¹;¹Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden, ²University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania, ³Department of Rheumatology, Karolinska Institutet, Stockholm, Sweden, ⁴Ecole Polytechnique Fédérale de Lausanne, Swiss Institute for Experimental Cancer Research, Switzerland.

Background and aims: Notch signaling is central for cell differentiation and angiogenesis. It is a cell-cell system composed of several Notch receptors (Notch1-4) and their ligands (Jagged 1-2, delta 1, 3, 4). The signaling is activated after binding of the ligands to the receptor which is followed by proteolytic cleavage of the receptor by γ -secretase complex with specific outcome

depending on the members of the Notch system involved. Diabetic wounds are characterized by impaired coordination of several cellular processes such as angiogenesis and cell differentiation. We have studied the potential role of Notch signaling in diabetic wound healing.

Materials and methods: Human dermal fibroblasts (HDF) and human dermal endothelial cells were used for *in vitro* studies and several animal models of diabetes (db/db mice and streptozotocin-induced diabetic mice) were used for *in vivo* studies. The functional consequence of the notch system modulation was studied *in vitro* by assessment of the migration of HDF and by angiogenesis assay. Notch pathway inhibition was induced either by γ -secretase inhibitors or by specific siRNA silencing of the Notch receptors (1–4). Using cre-lox system we have generated mice that lack Notch 1 in the skin. Wound healing rate was evaluated both in db/db mice and in skin-specific Notch1 knock-out mice in which diabetes was induced by streptozotocin.

Results: Notch signaling was activated in the skin of several animal models of diabetes and biopsies from patients with diabetic wounds. Hyperglycemia activated Notch pathway and had repressive effect on fibroblasts migration and angiogenesis. Mechanistically, we found that hyperglycaemia enhances delta4 expression in a Notch1-dependent manner and this positive delta4-Notch1 feedback loop contributed to the impaired wound healing in diabetes. This was confirmed *in vivo* where inhibition of Notch signaling with γ -secretase inhibitors improved wound healing rate just in diabetic (db/db mice) but not in control non-diabetic animals. Using loss-of-function genetic approaches we demonstrated both at the cellular level (fibroblasts, endothelial cells) as well as in an animal model that the Notch1 activation was the key player of the repressive effects of Notch on wound healing in diabetes, which was confirmed in the biopsies from patients with diabetic foot ulcers.

Conclusion: Glucose activates a positive feedback loop (delta4-Notch1) that contributes to the deleterious wound healing in diabetes.

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SP regulates macrophage function in diabetic wound healing

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Background and aims: Chronic diabetic foot ulcerations develop in areas affected by diabetic neuropathy. As consequence, neuropeptides, such as substance P (SP), known to modulate inflammation, showed to be important in wound healing. However, little it is known about the effect of SP on macrophages activation. We aimed to study the effect of SP on macrophage function in diabetic wound healing progression.

Materials and methods: We used wild type (WT) mice and two types of genetically modified mice: one deficient of the NK1 receptor of SP (NK1RKO) and one deficient of the TAC1 gene that encodes for SP and other takinins (TAC1KO). Also, we treated the wound with SP or CJ012,255 (CJ), an inhibitor of SP receptor. Diabetes was induced by streptozotocin intraperitoneal injection, 50 mg/Kg, 5 days. The animals were kept 8 weeks diabetic prior wound healing experiment. M1 and M2 macrophages were identified by using immunohistochemistry at several phases of wound healing: baseline (Day-0), Day-3 and Day-10 post-wounding. MCP-1, IL-a and KC was quantified by q-RT-PCR.

Results: The M1/M2 ratio was increased at Day-0 in WT-diabetic mice and nondiabetic and diabetic NK1R and TAC1 mice when compared to WT-nondiabetic mice. In WT-nondiabetic mice, the ratio increased at Day-3 but returned to normal levels by Day-10. In contrast, in WT-diabetic and nondiabetic and diabetic NK1R and TAC1 mice at Day-0, Day-3 and Day-10, the M1/M2 ratio remains high, suggesting a persistent inflammation. At Day-10, the M1/M2 ratio was increased in CJ- treated WT-nondiabetic and WT-diabetic mice. In contrast, SP treatment reduced the diabetes-induced increase in M1/M2 ratio. Similar results were observed in the skin gene expression of the monocyte chemoattractant 1 (MCP-1) that recruits monocytes in areas of inflammation where they are converted to macrophages. At Day-0, MCP-1 was increased in the TAC1KO mice. MCP-1 expression increased at Day-3 in all non-diabetic and diabetic mice. At day-10, all mice groups had a decreased MCP-1 expression but, nonetheless, it was increased in WT-diabetic. SP treatment increased MCP-1 expression at Day-3 in both WT-nondiabetic and diabetic wounds. At Day-10, SP treatment reduced MCP-1 expression in both WT-nondiabetic and diabetic mice while CJ-treated mice had higher expression when compared to SP-treated mice. Moreover, diabetic mice

showed an increase in IL-6 expression at baseline while SP induced a further increase in IL-6 gene expression in WT-nondiabetic mice at Day-3. NK1RKO and TAC1KO mice showed an increase in IL-6 expression at baseline. KC gene expression was also increased in WT diabetic mice at baseline and this increase persisted at Day-10. SP induced an acute increase at Day-3 that was followed by a reduction to normal levels at Day-10. NK1RKO and TAC1KO mice had also higher KC gene expression at baseline and NK1RKO mice showed a higher KC expression at Day-3.

Conclusion: In conclusion, the reduction in SP availability results in a pro-inflammatory activation of skin macrophages before wounding. SP also plays a major role in shifting macrophages to the M2 activation promoting wound healing. Furthermore, SP induced an increase in the acute inflammatory phase of wound healing during the early stages of wound healing but considerably reduces it in later stages, which is essential for a good skin repair.

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Living human keratinocytes as a therapeutic option to improve diabetic ulceration

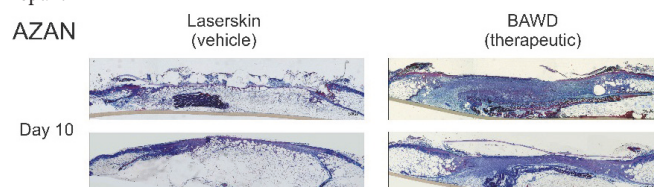
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Background and aims: Diabetes-associated foot ulcerations (DFU) represent a serious clinical complication of wound healing. To date, pharmacological approaches to improve diabetic wound healing disorders still remain poor and limited. The lack of knowledge for underlying molecular and cellular mechanisms of impaired wound healing particularly adds to this unsatisfactory clinical condition. Here we used human keratinocytes (BAWD, bio-active wound dressing) as a dynamic approach to deliver a living source of multiple wound-induced mediators for treatment of highly disturbed healing conditions in severely diabetic mice.

Materials and methods: BAWD consists of human keratinocytes cell line (KCBI1). The cells were cultured on a hyaluronic acid matrix. The KCBI1 cell line represents well-characterized, safe, and highly proliferative keratinocytes, which have been initially isolated from human foreskin. For wound healing studies, six full-thickness wounds (5 mm in diameter, 3–4mm apart) were made on the back of severely diabetic C57BL/6J-db/db mice. Upon injury, mice received a coverage of skin wounds using hyaluronic acid matrix alone (vehicle) or living keratinocytes (BAWD, therapeutic). Skin biopsy specimens were obtained from the animals at day 3, 7 and 10 after injury. Protein and RNA isolation was performed and wound biopsies were fixed for histology. The transcriptome of hyaluronic acid- and BAWD-covered wound tissue was analyzed by direct mRNA sequencing. Paraffin sections (4µm) were stained for collagen deposition (azan trichrome) or incubated using antisera against CD31, VEGF or myosin heavy chain 7.

Results: BAWD strongly improved diabetes-disturbed wound healing. BAWD-covered wounds showed a very robust formation of granulation tissue in the presence of hyperglycaemia. New tissue was characterized by high expression levels of angiogenic VEGF. Induced expression of the endothelial marker Tie-2 and a high density of newly formed blood vessels defined newly formed tissue. Moreover, gene expression analyses of BAWD-improved wound tissue showed a keratinocyte-mediated induction of a large set of genes, which are known to be specifically involved in skeletal and heart muscle development, differentiation and function. Improved wound tissue upon BAWD-treatment also showed combined expression levels of the mesenchymal stem cell markers Sca-1 and CD29.

Conclusion: BAWD markedly improved wound healing in diabetic mice by accelerating the formation of new tissue at sites of diabetes-impaired wounds repair.



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OP 46 New approaches and clinical outcomes of islet or pancreas transplantation

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Macro-encapsulated human embryonic stem cell-derived implants meet characteristics of clinical human islet cell grafts

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Background and aims: Shortage of good quality human pancreases for use in organ and islet cell transplantation has led to development of large-scale laboratory sources that generate insulin-producing implants. Pancreatic endocrine cells can be formed from human embryonic stem (huES) cells following in vitro derivation to pancreatic endoderm (PE) and further differentiation in immune-incompetent mice. This study examines to which extent huES-generated implants meet characteristics of human islet cells as used in clinical transplantation.

Materials and methods: Free, alginate-micro- and Theracyte-macro-encapsulated huES-derived PE-grafts of similar size were implanted in mice with severe-combined-immune-deficiency. Their in vivo differentiation was compared through plasma human C-peptide levels and cellular analysis of implants at posttransplant week 20. Implants retrieved from Theracyte-macro-devices were analyzed for glucose-regulated hormone synthesis and release. Data were compared with those collected for human islet cells.

Results: Endocrine enrichment was higher in encapsulated than free hu-ES-implants or in clinical-grade human islet cell grafts, with enrichment in single hormone-positive alpha, beta and delta cells in macro-devices and alpha cells in microcapsules. Macro-huES-implants resulted in higher plasma human C-peptide than free human islet cell transplants with similar cell number. They exhibited equally rapid insulin- and glucagon-secretory responses to increasing and decreasing glucose concentrations during perfusion. Their insulin secretory amplitude to glucose was lower, in part attributable to a lower cellular hormone content; this was associated with lower glucose-induced insulin biosynthesis, but not with lower glucagon-induced stimulation of release.

Conclusion: Macro-encapsulated huES-derived implants can reach an endocrine composition and function with therapeutic potential. Their beta cells exhibit rapid glucose responsiveness with lower amplitude, compatible with an immature functional state. Comparative data with clinical-grade human islet cell grafts set references for further development and clinical translation. *Supported by: EC-FP7, JDRF encapsulation consortium, Flemish Government, FWO*

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Impaired processing of human pro-islet amyloid polypeptide (proIAPP) promotes early islet graft failure

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Background and aims: Islet transplantation is a promising therapy for type 1 diabetes. Long-term survival of islet grafts may be compromised not only by immune rejection but also other factors including the formation of islet amyloid from islet amyloid polypeptide (IAPP). IAPP aggregation has been linked to beta-cell dysfunction, apoptosis, and islet inflammation but the underlying mechanism of islet amyloid formation remains elusive. Elevated circulating proinsulin:insulin ratios have been detected in type 2 diabetes and in type 1 diabetic recipients of islet transplants. Processing of the IAPP precursor, proIAPP, may be similarly compromised in islet transplants and type 2 diabetes, since like proinsulin, proIAPP is processed by the beta cell prohormone convertases PC1/3 and PC2. We hypothesize that accumulation of proIAPP intermediates leads to amyloid formation and beta-cell dysfunction and assessed the impact of impaired proIAPP processing in transplanted islets.

Materials and methods: NOD.SCID mice (age 8–10 weeks) were made diabetic with streptozotocin and transplanted with islets from mice with beta-cell expression of human proIAPP and lacking PC2 (hIAPP^{Tg0}; PC2^{-/-}) or

controls (hIAPP^{Tg0};PC2^{+/-}, hIAPP^{0/0};PC2^{-/-}, hIAPP^{0/0};PC2^{+/-}). Blood glucose was monitored weekly in recipients for 16 weeks or until graft failure (return of hyperglycaemia). Islet graft sections were stained for insulin, amyloid deposition (thioflavin S) and TUNEL positivity.

Results: Diabetes (blood glucose >16.9 mM) returned in almost all (90%) of recipients of hIAPP^{Tg0};PC2^{-/-} islets within 16 weeks post-transplant, whereas recipients with normal human proIAPP processing (hIAPP^{Tg0};PC2^{+/-}) remained normoglycemic for the entire 16 weeks. Recipients of grafts expressing native rodent IAPP, with or without PC2, similarly maintained normoglycemia. Despite early graft failure in recipients of islets with impaired human proIAPP processing, beta-cell mass remained unchanged and amyloid deposition was lower compared to human proIAPP-expressing mice with normal processing. Islet grafts had few TUNEL-positive beta cells and no difference in the number of TUNEL-positive cells among groups, suggesting that impaired human proIAPP processing compromises beta-cell function rather than promoting beta-cell apoptosis.

Conclusion: Impaired human proIAPP processing in islet transplants induces early graft failure without affecting beta-cell mass or increasing amyloid formation. These data suggest that impaired proIAPP processing promotes dysglycemia, possibly via the formation of early (pro)IAPP aggregates that induce beta-cell dysfunction. Our work implicates IAPP precursors as potential targets to prolong islet graft survival.

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In type 1 diabetic patients pancreas transplanted simultaneously with kidney preserves long-term kidney graft ultrastructure better than kidney transplantation alone

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Background and aims: In type 1 diabetic patients with end-stage renal disease (ESRD) we aimed to determine whether long-term normoglycaemia, as achieved by successful simultaneous pancreas and kidney (SPK) transplantation, would preserve kidney graft structure, determined by morphometry and electron microscopy (EM), and function compared with live donor kidney (LDK) transplantation alone.

Materials and methods: Estimated GFR (eGFR) was calculated in 25 recipients with SPK and 17 recipients with LDK in a stable phase 3 months after transplantation and annually during follow-up. Ultrasound guided kidney graft biopsies were obtained from all participants at follow-up. All biopsies were processed for light microscopy to measure glomerular volume, and EM to obtain data on glomerular basement membrane (GBM) width, mesangial fractional volume and podocyte coverage of the capillary basement membrane.

Results: SPK and LDK recipients were similar in age and diabetes duration at engraftment. The median duration of follow-up was 10.1 years. HbA1c was 5.6±0.4 % and 8.3±1.7 % in the SPK and LDK group, respectively (p<0.001). Compared with SPK recipients, patients who received LDK had significantly wider GBM (368.9±109.4 nm vs 281.0±56.7 nm; p=0.008) and increased mesangial fractional volume (23 (13, 59) % vs 16 (10, 41) %; p=0.006) at follow-up. Podocyte coverage of the capillary basement membrane was equal in the SPK (88 (58, 100) %) and LDK (89 (57, 100) %) group (p=0.35). Glomerular volume tended to be higher in LDK (4.5 (2.2, 8.2) × 10⁶ μm³) compared with SPK (3.2 (1.6, 12.9) × 10⁶ μm³) recipients (p=0.10). In the SPK group the eGFR declined from 78.3±16.7 at transplantation to 67.3±23.7 ml/min/1.73 m² at follow-up (-11.0±21.4 ml/min/1.73 m²), and the recipients of LDK declined from 68.9±13.2 to 46.2±14.2 ml/min/1.73 m² (-22.7±15.2 ml/min/1.73 m²), respectively (p=0.060).

Conclusion: In type 1 diabetic patients with long-term normoglycaemia after successful SPK transplantation kidney graft ultrastructure was better preserved compared with LDK.

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Coronary artery disease in type 1 diabetic patients long-term after simultaneous pancreas and kidney transplantation compared with kidney transplantation aloneT. Jenssen^{1,2}, J.P.H. Lindahl¹, A. Hartmann¹, K. Endresen³, A. Günther⁴;¹Department of Transplant Medicine, Oslo University Hospital,²University of Tromsø, ³Department of Cardiology,⁴Department of Radiology, Oslo University Hospital, Norway.

Background and aims: Improved long-term glycaemic control protects against development of cardiovascular disease and death in type 1 diabetic patients. We aimed to determine whether long-term normoglycaemia, as achieved by successful pancreas and kidney transplantation (SPK), would change the development of coronary artery disease (CAD) compared with successful live donor kidney transplantation alone (LDK).

Materials and methods: Twenty type 1 diabetic patients who had received SPK grafts were compared with 11 recipients of LDK. All patients included underwent baseline (pre-transplant) and follow-up coronary angiography. Computed tomography (CT) examination was performed at follow-up to obtain coronary artery calcium scoring (CACS). Logistic regression was used to evaluate association between CACS and CAD.

Results: Recipients of SPK grafts and LDK were similar in terms of age and diabetes duration at engraftment. The median duration of follow-up was 9.2 years. HbA_{1c} was 5.6±0.4 % and 8.4±2.0 % in the SPK and LDK group, respectively ($p<0.001$). In the SPK group the eGFR declined from 81.6±14.8 at transplantation to 69.9±22.0 ml/min/1.73 m² at follow-up (-11.7±22.0 ml/min/1.73 m²), and the recipients of LDK declined from 70.1±14.5 to 50.8±11.6 ml/min/1.73 m² (-19.3±11.9 ml/min/1.73 m²), respectively ($p=0.22$). Coronary angiography at baseline revealed that 11 out of 20 SPK recipients had CAD, defined as coronary luminal stenosis of 50 % or more in at least 1 vessel, compared with 1 out of 11 recipients in the LDK group ($p=0.020$). During follow-up there was no difference in patients with progression (defined as a new stenosis at or above 50 % in one or more vessels) of CAD between the SPK group (10 out of 20 recipients) and the LDK group (5 out of 11 recipients; $p=0.81$). Median Agatston scores of coronary arteries with CT scan at follow-up were extremely high: 1625 (9, 7368) and 1391 (4, 5897) for the SPK and LDK group, respectively ($p=0.92$). There was, on the other hand, no significant association between high CACS and coronary artery lumen narrowing (≥50% diameter stenosis) in the SPK group (OR 1.001, CI 1.000, 1.003; $p=0.079$) or in the LDK group (OR 1.000, CI 0.999, 1.001; $p=0.61$).

Conclusion: This study suggests that median 9 years of normoglycaemia, as achieved by successful SPK transplantation, is not sufficient to halt or reverse CAD in previously uraemic diabetic patients. Calcification of coronary arteries is a prominent feature in this group of patients.

OP 47 Pharmacogenetics and disease progression

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Modelling the rate of deterioration of diabetes in observational data: a DIRECT studyL.A. Donnelly¹, K. Zhou¹, C. Jennison², E.R. Pearson¹;¹Division of Cardiovascular & Diabetes Medicine, University of Dundee,²Department of Mathematical Sciences, University of Bath, UK.

Background and aims: The rate at which diabetes progresses following diagnosis of type 2 diabetes (T2D) is highly variable between individuals. Outwith clinical trials of monotherapy such as ADOPT and UKPDS, it is challenging to model progression rates in the population of patients with diabetes due to the fact that underlying 'diabetes severity' reflects both drug treatment and HbA_{1c}. The aim of this study was to combine these measures to derive a Diabetes Severity Score for each individual and then model the rate of progression.

Materials and methods: We utilised the electronic medical records from patients in the Genetics of Diabetes Audit and Research (GoDARTS) cohort, Tayside, Scotland. A Diabetes Severity Score was derived based on a patient's observed HbA_{1c} measures from diabetes diagnosis through to study end (defined as earliest of insulin initiation, death or 31st July 2011). We expected patients to exhibit a steady progression and this would appear as a gradual increase in Diabetes Severity Score. We therefore fitted a linear model with a slope and intercept for each individual, an additional parameter for every drug combination, BMI category and an error term.

Results: 3533 patients were included in the analysis with a median follow up time of 7.5 years and 17 HbA_{1c} measures. Mean age at diagnosis was 63 years and 56% were male. 12% patients progressed to insulin treatment over the follow-up period. The estimated effect on HbA_{1c} of the diabetes drugs was in line with clinical trial data. For example Metformin lowered HbA_{1c} in a dose dependent manner (-0.21%<500mg; -0.35% 500mg; -0.49% 850mg; -0.61% 1G; -0.8%>1G) and sulphonylureas had a dose independent effect of -0.52%. The median progression rate was an increase in 0.1% HbA_{1c} per year. The top 25% of progressors had an increase >0.3% and the top 10% >0.6%. The lower 25% had little or no progression. When comparing the highest vs lowest quartile, predictors of rapid progression at baseline were younger age at diagnosis (mean 58.2 vs 67 years, $p<0.0001$); lower HDL (1.16 vs 1.24 mmol/l, $p<0.0001$); higher triglycerides (2.71 vs 2.39 mmol/l, $p<0.0001$); higher BMI (32.3 vs 31.3 kg/m², $p=0.0002$) and; higher HbA_{1c} (8.02 vs 7.75%, $p=0.0015$). In addition 34.1% of the highest quartile progressed to insulin vs 1.6% in the lowest.

Conclusion: We have developed a novel approach to model diabetes progression in observational data across multiple drug combinations. The progression rates are similar to that described for monotherapy in ADOPT (0.14% HbA_{1c} per year in metformin group); with progression faster in those with a more obese insulin resistant phenotype. This approach can be used to investigate biological determinants of progression as well as the impact of drug effects on progression rates. Gaining insight into why some patients progress rapidly and some do not will enable a more stratified approach to the management of T2D.

Supported by: IMI

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HbA_{1c} trajectories in type 2 diabetes patients: The Diabetes Care System cohort

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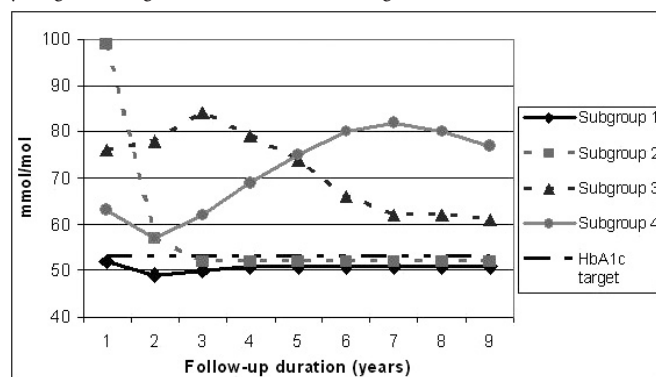
Background and aims: The revised ADA/EASD guidelines for the management of type 2 diabetes mellitus (T2DM) recommend HbA_{1c} levels of ≤ 53 mmol/mol. However, clinical characteristics of T2DM patients with glycaemic control trajectories that are distinct from achieving the recommended HbA_{1c} target are inadequately studied. In order to eventually improve glycaemic control, the primary aim of this study was to determine subgroups of T2DM patients with distinct trajectories of HbA_{1c} levels. Furthermore, sub-

group characteristics were determined and the prevalence of microvascular complications over time was investigated.

Materials and methods: 5423 T2DM patients with at least two HbA1c follow-up measurements were selected out of 9849 T2DM patients from the primary Diabetes Care System cohort. The mean follow-up period was 5.7 years (range 2 to 9 years). Latent Class Growth Modelling (LCGM) was performed to identify subgroups of patients with distinct trajectories of HbA1c levels. Multinomial logistic regression analyses were conducted to determine subgroup characteristics. Associations of different subgroups with retinopathy, microalbuminuria and medication use during follow-up were studied by constructing plots and by graphically comparing differences between subgroups.

Results: Four subgroups with distinct trajectories of HbA1c levels were identified (Figure). The first and largest subgroup (83%) maintained good glycemic control over time (HbA1c ≤ 53 mmol/mol), the second subgroup (8%) initially showed severe hyperglycaemia, but reached the recommended HbA1c target within 2 years. Patients within this subgroup had significantly higher baseline HbA1c levels but were otherwise similar to the good glycemic control subgroup. The third subgroup (5%) showed hyperglycaemia and a delayed response without reaching the recommended HbA1c target. The fourth subgroup (3.0%) showed deteriorating hyperglycaemia over time. Patients within the last two subgroups were significantly younger, had higher baseline HbA1c levels and had a longer diabetes duration at baseline. These subgroups also showed a higher prevalence of retinopathy and microalbuminuria compared to the good glycemic control subgroup.

Conclusion: This is the first study identifying subgroups with distinct trajectories of HbA1c levels in a T2DM cohort. More than 90% of the patients reached and maintained good glycemic control. Two subgroups showed a more unfavorable course of glycemic control. These T2DM patients were younger, had higher HbA1c levels and a longer diabetes duration at baseline.



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Statistical power enhancement in diabetic drug trial by selectively sampling participants from the tails of biomarker distributions: a DIRECT study

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Background and aims: Within the IMI DIRECT Study we are seeking to identify biomarkers that modify treatment response, primarily using observational studies to generate hypotheses that will be validated in clinical trials. Here, we compared statistical power to detect gene-metformin interactions in two types of trials: approach 'A' represents interactions evaluated in an existing clinical trial that involves randomly selected participants; approach 'B' involves selecting participants on the basis of discordant levels of genetic risk score (GRS) comprised of 20 variants that modify treatment effects (genotype-based recall: GBR).

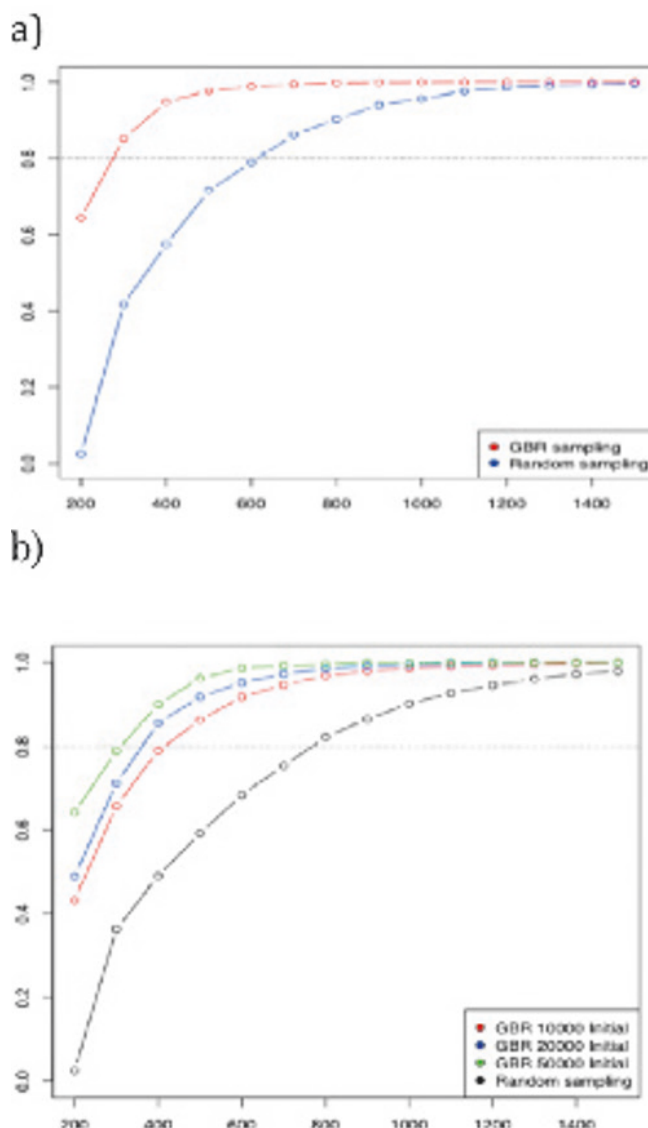
Materials and methods: Model assumptions were based on published data from the Diabetes Prevention Program, a randomized controlled trial of metformin treatment vs. placebo. We simulated GRS-metformin interactions, assuming the 20 GRS variants convey comparable gene-metformin interaction effects to those reported for the MATE1 locus. The effects of the metformin and placebo interventions were estimated assuming randomised treatment allocation. To simulate approach 'A', participants were randomly sampled

from a larger population-sampling frame (N=10,000-50,000), as might be the case in a conventional clinical trial. To simulate approach 'B', participants were purposefully sampled at random and from the upper tail of GRS distribution from the same sampling frames. Time to diabetes event was calculated using the proportional hazards function. Sample size calculations were performed using 1000 iterations per simulation in the R software package.

Results: Figure 1 shows representative results from these analyses: to obtain 80% power to detect the specified gene x metformin interaction effect, the GBR approach would require 300-400 participants, whereas the random-sampling approach would require roughly 800 participants for rare alleles (Fig. 1a). For common alleles a sample size of ~250 would suffice with the GBR approach compared with sample size of ~650 for the random sampling approach. Similarly, where interaction effects are smaller in magnitude, the difference in sample-size requirements for the random vs. the GBR sampling approach increases substantially. The size of the sampling-frame also affects power (Fig. 1b).

Conclusion: The GBR approach for validating observations of gene-drug interaction offers a potentially powerful alternative to conventional clinical trials, which is especially appealing when gene variants are rare and phenotyping is expensive.

Figure 1:



Supported by: EC and EFPIA.

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Antidepressant medication use and trajectories of fasting plasma glucose and HbA_{1c} levels: a 9-year longitudinal study of the DESIR cohort

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Background and aims: Some research has suggested that antidepressant medication use (AMU) may increase the type 2 diabetes (T2D) risk. However the causal mechanism of this association remains unclear. To study the biological plausibility of this relationship, we examined the association between AMU and changes in fasting plasma glucose (FPG) and in HbA_{1c} levels over time.

Materials and methods: Participants were 4869 men (49.2%) and women, free of T2D and aged 30–65 years at baseline, followed for 9 years between 1994 and 2005 in the French D.E.S.I.R. cohort study. AMU, FPG and HbA_{1c} were assessed concurrently at 4 medical examinations in phases 1 (1994–1996), 2 (1997–1999), 3 (2000–2002) and 4 (2003–2005). Linear mixed models were used to examine the longitudinal associations of AMU with FPG and HbA_{1c} levels.

Results: FPG and HbA_{1c} levels increased over follow-up. In model adjusted for sociodemographic characteristics, health-related behaviours, medical conditions and other medications, there was no difference in mean FPG and HbA_{1c} levels ($\beta = -0.001$ $p = 0.974$, $\beta = 0.012$ $p = 0.662$, respectively) among antidepressant medication users and non-users at baseline (1994–1996). The interaction term between time and AMU, suggested no increase in FPG and HbA_{1c} levels in antidepressant users comparatively to non-used over 9-year of follow-up ($\beta = -0.004$ $p = 0.535$, $\beta = -0.002$ $p = 0.548$ respectively). Similar patterns of associations were observed when the type and the cumulative use of antidepressants over follow-up were considered.

Conclusion: Our results suggest that AMU is not associated with an increase in FPG and HbA_{1c} levels over 9-year of follow-up. This suggests that the nature of the association between AMU and T2D risk, observed in previous studies, may not be causal.

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OP 48 Novel targets for anti-inflammatory therapies

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Exogenous adiponectin administration through a subcutaneous minipump reverses high-fat diet-induced impairment of adipose tissue metabolism

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Background and aims: Despite adiponectin administration is thought to be a future strategy to obese or type 2 diabetic patients, it still is a myth, due to its expensive costs and absence of studies demonstrating the effectiveness of chronic exogenous adiponectin. Our aim was to develop a method to produce adiponectin for long-term adiponectin administration and test its usefulness in improving metabolic profile and adipose tissue metabolism in high-fat diet fed rats.

Materials and methods: Adiponectin (98 µg/day) was administered during 28 days through a subcutaneous minipump with continued release to normal Wistar rats fed a high-fat diet. Several systemic markers of dysmetabolism and pathways of insulin signaling and lipid storage in epididymal and subcutaneous adipose tissue were assessed.

Results: Exogenous adiponectin was able to decrease body weight, fasting glycaemia, HbA_{1c} and cholesterol levels (total and non-HDL). In adipose tissue, adiponectin reverted high-fat diet-induced impairment of insulin signaling and decreased IκBα and PPARγ levels, which were mainly observed in epididymal adipose tissue. High-fat fed rats showed little activation of lipolysis during fasting in epididymal adipose tissue, despite no alterations were observed in the total amount of the protein. This was only partially reverted by adiponectin.

Conclusion: Long-term adiponectin administration through a subcutaneous minipump was able to improve pathways of insulin signaling and lipid storage in adipose tissue after the consumption of a high-fat diet. Adiponectin was also able to improve the metabolic profile, probably as a result of improved adipose tissue metabolism.

Supported by: FCT

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Immuno-neutralisation of extra-cellular nicotinamide phosphoribosyltransferase as a therapeutic strategy for treatment of type 2 diabetes

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Background and aims: The protein extra-cellular nicotinamide phosphoribosyltransferase (eNAMPT; also termed visfatin/PBEF) is elevated in serum of type 2 diabetes (T2D) patients and raised eNAMPT levels are reported to strongly correlate with declining beta-cell function. Moreover, eNAMPT reportedly exerts pro-inflammatory effects suggesting a potential pathophysiological role for eNAMPT in T2D. However, the precise functional relationship between elevated serum eNAMPT and the pathophysiology of T2D has not been determined. Interestingly, eNAMPT exists in serum as a monomer and a dimer, suggesting a potential structure/function relationship, although the precise function and specific changes in each form in T2D have not been determined.

Materials and methods: To investigate the role of eNAMPT in T2D, C57Bl/6 mice were fed a high-fat diet (HFD; 60% fat) for 10 weeks. In weeks 9–10, mice were administered polyclonal anti-NAMPT antibody (2.5 µg/ml; 2 doses/week) or saline equivalent. After week 10, mice were fasted for 16 h prior to glucose tolerance testing (2 g/kg body weight glucose) or sacrificed in the fed state for tissue (islets, liver and white adipose tissue; qPCR and western blot) and serum analysis.

Results: Serum eNAMPT monomer levels were elevated in HFD mice, whilst dimer levels were unchanged, as assessed by Native-PAGE immunoblot. Immuno-neutralization of eNAMPT corrected HFD-mediated fasting and fed hyperglycaemia, hyperinsulinemia and impaired glucose tolerance. These

effects were mediated through improvements in beta-cell health (increased ex vivo and in vivo islet glucose-stimulated insulin secretion and increased islet size) and correction of hepatic insulin resistance (reduced gluconeogenic gene expression, increased insulin signalling). Consistent with a pro-inflammatory function of eNAMPT, improvements in glycaemia and beta-cell health following eNAMPT-immuno-neutralization were linked to lowering of HFD-mediated increases in gene expression of islet and white adipose tissue pro-inflammatory cytokines, chemokines and immune cell markers, as well as decreased mRNA levels of islet pro-apoptotic markers.

Conclusion: Elevated serum eNAMPT monomer levels may contribute to pathophysiology of T2D, through pro-inflammatory effects. Moreover, selective neutralization or inhibition of eNAMPT monomer represents a potential therapeutic target for treatment of T2D.

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TLR4 inhibition blocks cytokine production and restores beta cell survival in human pancreatic islets

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Background and aims: Inflammatory signals are strong mediators of metabolic failure in fat, liver, brain, muscle as well as in pancreatic islets. Toll-like receptor-4 (TLR-4) signaling is one of the major pro-inflammatory pathways, whose ligands as well as downstream products, i.e. cytokines and chemokines, are increased systemically in patients with T2D as well as in at-risk individuals. TLR4 knockout mice are protected from the metabolic consequences of a high fat diet. In the present study we investigated the consequences of TLR4 activation in human islets and whether pharmacological inhibition of TLR4 restores beta-cell survival in a diabetogenic milieu.

Materials and methods: Isolated human islets were exposed to the TLR4 ligand Lipopolysaccharide (LPS) in the presence or absence of TLR4 receptor small molecule antagonist, TAK-242 (Resatorvid). The effect of TLR4 inhibition was compared with those achieved by the depletion of islet resident macrophages by treatment of human islets with clodronate liposomes. Expression and secretion of pro-inflammatory cytokines/chemokines were evaluated by RT-PCR and ultrasensitive ELISA. TLR4 downstream signaling activation and apoptosis were analyzed by Western Blotting.

Results: Treatment of isolated human islets with LPS elevated mRNA levels of pro-inflammatory cytokines/chemokines, including IL-1alpha, IL-1beta, IL-6, TNFalpha, CCL2 and IL-8. LPS induced IL-1beta secretion was confirmed by ELISA. TAK-242 completely blocked LPS-induced cytokine/chemokine expression and IL-1β secretion, while clodronate treatment only inhibited IL-1alpha/beta but not IL-6, IL-8, TNFalpha and CCL2 expression. TAK-242 could completely antagonize the effects of LPS on TLR-4 downstream signaling and apoptosis in human islets, it inhibited phosphorylation of inhibitor of nuclear factor kappa B alpha (IκBα) and c-Jun N-terminal kinase (JNK) as well as caspase-3 cleavage.

Conclusion: Our results show that TLR4 activation in human islets leads to islet inflammation and beta-cell apoptosis, which results in beta-cell failure, while the TLR4 inhibitor TAK-242 inhibits cytokine production and restores beta-cell survival.

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Intervention with Caspase-1 inhibitor attenuates the metabolic syndrome and prevents non alcoholic-steatohepatitis (NASH) in high fat diet-fed LDLR-/-Leiden mice

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a serious liver pathology which develops as a complication of the metabolic syndrome. Recently, the inflammasome was proposed to be involved in the development of NASH. Here we investigate whether a chemical inhibitor of caspase-1 in already manifest metabolic syndrome would prevent the development to NASH.

Materials and methods: Male LDLR-/-Leiden mice were fed a high fat diet (HFD; group 1) or low fat diet (LFD; group 2) for 21 weeks. In a third group, intervention with caspase-1 inhibitor Ac-YVAD-CMK (40 mg/kg daily) was started after 9 weeks of HFD (manifest metabolic syndrome) and continued until 21 weeks.

Results: HFD treated mice developed obesity and insulin resistance after already 9 weeks of HFD feeding. Intervention with caspase-1 inhibitor attenuated a further development of insulin resistance, and reduced body weight gain as well as adipose tissue inflammation compared to HFD. Histopathological analysis of the livers clearly demonstrated prevention of NASH development with caspase-1 inhibitor: livers were less steatotic and neutrophil infiltration was diminished. Additionally, hepatic fibrosis quantified by sirius red staining and acta2 and col1a1 gene expression as observed in HFD treated mice, was completely prevented.

Conclusion: Intervention with a caspase-1 inhibitor in already established disease improved hallmarks of the metabolic syndrome and prevented the development of NASH. Our data further support the importance of caspase-1/inflammasome in the development of NASH and demonstrate that therapeutic intervention in the already ongoing disease process is feasible.

PS 001 Pragmatic prediction and prevention of type 2 diabetes

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Family history of diabetes is a strong predictor of diabetes, hypertension and metabolic syndrome in Sri Lankan adults

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Background and aims: Diabetes mellitus has become an important health concern in South Asia. Family history (FH) is a common non-modifiable risk factor for most of the chronic non-communicable diseases, as it is a collective reflection of the genetic susceptibility, shared environments and behaviors. Information on FH may serve as a unique and useful tool for public health and preventive medicine. The increased genetic predisposition amongst South Asians probably makes FH more important in risk assessment than in other ethnic groups. The present study aims to describe the influence of FH on diabetes prevalence and associated metabolic risk factors in a nationally-representative sample of South Asian adults from Sri Lanka.

Materials and methods: A cross-sectional community based national survey was conducted in 7 of the 9 provinces in Sri Lanka. Five thousand adults were recruited for the study using a multi-stage stratified cluster sampling technique. An interviewer administered questionnaire was used to collect data, which included; age, gender, area of residence, ethnicity, level of education, household monthly income, duration of diabetes, FH, height, weight, waist circumference, hip circumference and blood pressure. FH was evaluated at three levels, a) parents, b) grandparents (paternal and maternal) and c) siblings. Presence of diabetes in children was also evaluated. A binary-logistic regression analysis controlling for confounders (age, gender, body mass index and physical activity) was performed in all patients with 'presence of diabetes' as the dichotomous dependent variable and using FH in father, mother, maternal grandmother/grandfather, paternal grandmother/grandfather, siblings and children as binary independent variables. A p value <0.05 was considered significant.

Results: Sample size was 4485, mean age was 46.1±15.1 years and 39.5% were males. The crude prevalence of diabetes, hypertension and metabolic syndrome were 12.0%, 27.2% and 26.6% respectively. The overall prevalence of FH in the population was 25.5%. In all adults the prevalence of diabetes was significantly higher in patients with a FH (23.0%) than those without (8.2%) (p<0.001). The prevalence of diabetes also increased with the number of affected generations (one - 33.9%, two - 34.2% and three - 37.1%). Presence of a FH significantly increased the risk of diabetes (OR:3.35,95%CI:2.78-4.03), obesity (OR:2.45,95%CI:1.99-2.99), hypertension (OR:1.25,95%CI:1.08-1.45) and metabolic syndrome (OR:2.28,95%CI:1.97-2.63). In all adults presence of FH in father (OR:1.29,95%CI:1.02-1.63), mother (OR:1.23,95%CI:1.11-1.36), paternal grandfather (OR:1.27,95%CI:1.14-1.41), siblings (OR:4.18,95%CI:3.34-5.22) and children (OR:5.47,95%CI:2.93-10.19) all were associated with significantly increased risk of developing diabetes.

Conclusion: FH and diabetes had a graded association in the Sri Lankan population, as the prevalence increased with the increasing number of generations affected. FH of diabetes was also associated with the prevalence of obesity, metabolic syndrome and hypertension. Individuals with a FH of diabetes form an easily identifiable group who may benefit from targeted intervention to prevent the development of diabetes and related metabolic disease. Supported by: NSF Sri Lanka

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The FINDRISC scale substantially reduces its performance as a screening tool for diabetes if diagnosed by HbA1c instead of glucose

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Background and aims: Recently it has been suggested that progression to diabetes can be delayed by intensive interventions when applied to real-life primary health care of high-risk subjects identified first with the simple Finn-

ish Diabetes Risk Score (FINDRISC) tool. The aim of the study was to investigate differences in the performance of the FINDRISC as a screening tool for glucose abnormalities after shifting from glucose-based diagnostic criteria to the proposed new hemoglobin (Hb)A1c-based criteria.

Materials and methods: A cross-sectional primary-care study was conducted as the first part of an active real-life lifestyle intervention to prevent type 2 diabetes within a high-risk Spanish Mediterranean population. Individuals without diabetes aged 45-75 years (n = 3,120) were screened using the FINDRISC. Where feasible, a subsequent 2-hour oral glucose tolerance test and HbA1c test were also carried out (n = 1,712). The performance of the risk score was calculated by applying the area under the curve (AUC) for the receiver operating characteristic, using three sets of criteria (2-hour glucose, fasting glucose, HbA1c) and three diagnostic categories (normal, prediabetes, diabetes).

Results: Defining diabetes by a single HbA1c measurement resulted in a significantly lower diabetes prevalence (3.6%) compared with diabetes defined by 2-hour plasma glucose (9.2%), but was not significantly lower than that obtained using fasting plasma glucose (3.1%). The FINDRISC at a cut-off of 14 had a reasonably high ability to predict diabetes using the diagnostic criteria of 2-hour or fasting glucose (AUC = 0.71) or all glucose abnormalities (AUC = 0.67 and 0.69, respectively). When HbA1c was used as the primary diagnostic criterion, the AUC for diabetes detection dropped to 0.67 (5.6% reduction in comparison with either 2-hour or fasting glucose) and fell to 0.55 for detection of all glucose abnormalities (17.9% and 20.3% reduction, respectively), with a relevant decrease in sensitivity of the risk score.

Conclusion: A shift from glucose-based diagnosis to HbA1c-based diagnosis substantially reduces the ability of the FINDRISC to screen for glucose abnormalities when applied in this real-life primary-care preventive strategy. As far as we know, this is the first estimate of a possible loss of performance of the FINDRISC questionnaire if there is a widespread use of these new proposed HbA1c-based diagnostic criteria, at least as a screening tool in the context of a program aimed at preventing diabetes.

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Value of the FINDRISK questionnaire to identify prediabetes and undetected type 2 diabetes

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Background and aims: The prevalence rate of Type 2 diabetes mellitus (T2D) has been increasing worldwide. The associated cardiovascular risk factors are connected with the development of micro- and macrovascular complications in the course of the disease and sometimes even before the diagnosis of diabetes. T2D is preceded by a pre-diabetic state with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and, in many cases, the metabolic syndrome. In this state, which may last for many years, several studies have shown that diabetes can be prevented. Identification of subjects with prediabetes is therefore of great importance, so that preventive action aimed at reducing their risk of T2D and cardiovascular disease (CVD) can be offered. Due to cost-effectiveness, screening should primarily be implemented in subjects at high risk to abnormal glucose tolerance (AGT=IFG/IGT/T2D). Aim is to evaluate the FINDRISK questionnaire as a screening tool for diabetes and prediabetes in a Russian population. In addition, we analysed the association between FINDRISK and CVD risk factor levels.

Materials and methods: In a population-based screening for AGT, a total of 1366 adults were invited. Full data with risk score estimate and glucose tolerance status were available for 560 subjects without known diabetes. AGT was diagnosed using standard OGTT according to WHO 1999/2006 criteria. Body mass index (BMI), waist circumference (WC), hypertension, blood lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) were determined. To assess performance of the risk score with respect to T2D and AGT, the area under the receiver operating characteristic curve (AUC), sensitivity, specificity, positive and negative predictive values were calculated.

Results: The prevalence of screen-detected T2D was 6.1%, whereas 36.1% were classified as having AGT. There was a marked increase in the prevalence of T2D and AGT with increasing value of the risk score. With risk score > 10, the positive predictive values (PPV) for T2D and AGT were 11% and 48%, respectively. Corresponding prevalence were 15% and 59% at higher level > 15. The AUC for T2D was 0.766, p < 0.001, whereas for AGT the area under the curve was 0.663, p < 0.001. Using the optimal cut-off value of 10 with the largest area under the curve to identify previously undiagnosed patients with T2D, AGT resulted in a sensitivity of 91%, 67% and specificity of

52%, 59%. To achieve this, only 50% of the total population would need to be screened. Increasing the cutoff value of the score to 15 changed the sensitivity to 38%, 25% and specificity to 86%, 90% respectively. Apart from total cholesterol and LDL cholesterol, several risk factors for cardiovascular disease: age ($p < 0.001$), BMI ($p < 0.001$), WC ($p < 0.001$), systolic and diastolic blood pressure ($p < 0.001$), fasting and 2h plasma glucose ($p < 0.001$), triglycerides ($p = 0.016$), HDL cholesterol ($p = 0.003$) had a direct association with the FINDRISK values.

Conclusion: FINDRISC proved to be simple and effective test to screen subjects at high risk for T2D. With the optimal cut-off level, the FINDRISC identified 91% undetected T2D and 67% AGT with relatively high PPV 48% for AGT, but only 11% of PPV for new T2D.

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Trends in leisure time physical activity in Danish adults with diabetes

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Background and aims: During the last decades there has been an increased focus on physical activity and exercise training in treatment of diabetes. The primary aim of this study was to compare leisure time physical activity (PA) reported in 2000, 2005 and 2010 by Danish subjects with diabetes. Furthermore, we analysed the subjects' smoking, alcohol consumption, and body mass index (BMI).

Materials and methods: Data comprised level of leisure time PA in four categories: physical inactivity, moderate activity, medium activity, and high activity; smoking; alcohol consumption; and BMI provided by The Danish Health and Morbidity Surveys, nationwide surveys ($n \sim 15,000$) from the general population. Subjects older than 45 years at the time of the surveys were included from cross-sectional analyses from 2000, 2005 and 2010.

Results: The diabetes prevalence was 4.5% ($n=386$) in 2000, 6.0% ($n=489$) in 2005, and 7.5% ($n=622$) in 2010. In subjects with diabetes, percentages of inactive women decreased from 42.2% to 21.8% ($p < 0.001$) and inactive men from 32.2% to 20.9% ($p=0.01$), with elevated prevalence in the three ascending levels of PA (moderate, medium, or high active) from 2000 to 2010. Prevalence of daily smokers was reduced from 29.3% to 20.1% ($p=0.002$). Subjects who exceeded a recommended maximum of alcohol consumption were increased from 6.5% to 14.0% ($p=0.001$). The BMI increased from 27.3 ± 4.7 to 28.6 ± 5.5 kg.m⁻² in diabetes subjects ($p=0.001$). In subjects without diabetes, the prevalence of physical inactivity decreased in women from 20.9% to 11.9% ($p < 0.001$) and men from 17.5% to 11.4% ($p < 0.001$). The PA level was reduced in subjects with diabetes compared with subjects without diabetes throughout the study.

Conclusion: The percentage of physically inactive Danish subjects older than 45 years with diabetes decreased from 2000 to 2010 with corresponding increases of percentages of subjects with moderate, medium, and high levels of PA. The trends of increased level of PA and decreased smoking may have impact on cardiovascular risk and positively affect the declining relative mortality among Danish patients with diabetes as shown in previous studies. On the other hand, elevated alcohol intake and increased BMI may increase the cardiovascular risk. Even though the PA level was elevated among subjects with diabetes, it remained reduced compared with the reported level of PA among subjects without diabetes.

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Effect of high intensity interval training on physical fitness, metabolic flexibility and insulin sensitivity

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Background and aims: The purpose of this study was to evaluate the effect of high intensity interval training on anthropometric parameters, physical fitness, metabolic flexibility and insulin sensitivity in participants with overweight or obesity compared to continuous aerobic training with the same total volume.

Materials and methods: 16 male participants with overweight or obesity (age range: 42 - 57, body mass index: 28 - 36) were randomized in two experimental groups, high intensity interval training ($n=8$) and continuous aerobic training ($n=8$). Participants were excluded if they had diabetes, severe mus-

culoskeletal, cardiovascular and respiratory problems. High intensity interval training was composed of three blocks of 10 minutes at ventilatory threshold (blocks 1 and 3: 10 sprint bouts of 15 seconds, followed by 45 seconds relative rest; block 2: continuous training) twice a week for 15 weeks. Continuous aerobic training was composed of three blocks of 10 minutes continuous training. After 5 weeks, intensity was increased to 110% of ventilatory threshold. Before and after the training period, body composition, physical fitness (expressed as peakVO₂ and anaerobic threshold (AT)), metabolic flexibility (expressed as resting respiratory exchange ratio (rRER)) and insulin sensitivity by oral glucose tolerance test were evaluated. The OGTT-composite score was calculated as $(10,000/\text{square root of } [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean glucose} \times \text{mean insulin during OGTT}])$. Significance level was calculated based on non-parametric Kruskal-Wallis test with post hoc Mann-Whitney U-test and set at $P < 0.05$.

Results: High intensity interval training showed a significant positive evolution ($P < 0.01$) compared to continuous aerobic training for peak VO₂ (+18%), peak Wattage (+14%), ATVO₂ (+14%) and ATWattage (+21%) indicating an increased aerobic capacity. After 10 weeks, there was a significant decrease ($P < 0.05$) of rRER (interval: pre: 0.85; post: 0.79; continuous: pre: 0.87; post: 0.86), indicating a better metabolic flexibility and a significant increase ($P < 0.01$) of the OGTT composite score (interval pre: 1.67; post: 2.96; continuous pre: 2.21; post: 2.33), indicating an increased insulin sensitivity.

Conclusion: In this study we could observe that high intensity interval training has stronger beneficial effects on body composition, physical fitness, metabolic flexibility and insulin sensitivity compared to continuous aerobic training with the same total volume.

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A priori defined diet quality indexes and risk of type 2 diabetes mellitus: the multiethnic cohort

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Background and aims: Dietary patterns have been associated with diabetes incidence; however, little is known about the impact of ethnicity on this association. Our aim was to assess the association between predefined dietary indexes and risk of type 2 diabetes (T2D) among white, Japanese American, and Native Hawaiian men and women in the Hawaii component of the Multiethnic Cohort (MEC).

Materials and methods: After excluding participants with prevalent diabetes or missing values on covariates we used data from 41,918 men (5,791 incident cases) and 47,267 women (5,426 incident cases). Habitual dietary intake was assessed at baseline with a food frequency questionnaire designed for use in the relevant ethnic populations. Sex- and ethnic-specific hazard ratios and 95% confidence intervals were calculated using adjusted Cox models for four dietary indexes, the Healthy Eating Index-2010 (HEI-2010), the alternative HEI-2010 (AHEI-2010), the alternate Mediterranean diet score (aMed), and the Dietary Approaches to Stop Hypertension (DASH).

Results: We observed strong inverse associations of adherence to the DASH index and T2D risk in white men and women, as well as Japanese American women and Native Hawaiian men, with respective risk reductions of 37, 31, 19 and 21% comparing the categories of highest to lowest adherence to index (score point ranges in both sexes: 28-39 to 9-19). A higher adherence to AHEI-2010 was related with lower T2D risk in white men and women with a 26 and 22% lower risk comparing the categories of highest to lowest adherence to index (score point ranges, men: 73-101 to 25-56, women: 47-100 to 30-58), whereas higher adherence to aMed was associated with a 28% lower risk of T2D in white men only comparing the categories of highest to lowest adherence to index (score point ranges: 7-9 to 0-2). Neither the AHEI-2010 nor the aMed were related to T2D risk in other ethnic groups than whites. Adherence to HEI-2010 was not associated with T2D risk.

Conclusion: In this large multiethnic cohort, the DASH, the AHEI-2010, and the aMed indexes performed well in whites, whereas only the DASH index was associated with a reduction in T2D risk in Japanese American women and Native Hawaiian men. Given the possible ethnic differences, further studies are warranted that focus on dietary indexes and their performance in multiethnic and minority populations.

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Sex-specific differences in prevention of type 2 diabetes mellitus: a systematic review and meta-analysis

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Background and aims: Type 2 diabetes mellitus (T2DM) is becoming an increasing challenge worldwide. Lifestyle interventions and several glucose lowering medications have been proved to be effective in preventing the progression to T2DM in people with prediabetes. The ADA/EASD position statement 2012 claims a patient centred approach, in which sex and gender aspects are relevant aspects. Sex-specific differences of the efficacy and safety of diabetes prevention strategies have not been studied so far.

Materials and methods: A systematic search of PubMed, Cochrane, Embase, CINAHL Web of Science, and reference lists of review articles from 1980 to June 2013 was performed. Literature was dually reviewed and risk of bias was rated for each study. Only randomised controlled trials with at least 1-year follow-up that compared active lifestyle interventions with usual care and pharmacotherapy trials with glucose lowering drugs that compared active treatment with placebo were included. Random effects meta-analysis of outcomes of interest was performed.

Results: We identified 2548 relevant abstracts; 309 full-text articles were retrieved for further examination. Eighteen RCTs (44articles) met the eligibility criteria. Only 3 published sex-specific results; of 9 RCT's unpublished sex-specific data was supplied upon request. A similar efficacy of risk or harms of lifestyle interventions and pharmacological therapies between men and women was found. Lifestyle interventions reduced the risk to develop T2DM in prediabetic people in both sexes. After 1 year lifestyle intervention group had a lower risk than patients in control group (RR:0.60; 95% CI:0.35-1.05; 4RCTs,888patients). A 37% lower risk of progressing to T2DM for pre-diabetic people receiving an average of 3 years of lifestyle intervention compared with those under usual care (RR:0.63; 95% CI:0.51-0.79; 5RCTs,1555patients) was found. Similar preventive effects of lifestyle interventions were found in men and women after 1 and 3 years. ($p=0.61$, $p=0.20$). Only few studies provided sex-stratified results of pharmacological therapies in T2DM prevention. Long-term evidence on potential sex differences of diabetes-associated comorbidity and mortality is missing.

Conclusion: The evidence indicates that intensified lifestyle intervention in structured programs is equally effective in both sexes. Therefore individually designed interventions to increase physical activity and healthy nutrition by behavioral modification for effective diabetes prevention should be offered for both sexes. More studies to reveal sex and gender-specific differences are necessary to follow a more patient centred approach.

Clinical Trial Registration Number: PROSPERO 2012:CRD42012003102

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Beneficial effect of pitavastatin on the incidence of diabetes in women was not associated with age: sub-analysis of J-PREDICT

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Background and aims: Diabetes mellitus (DM) increases the risk of coronary heart disease (CHD) far more in women than men. If statin therapy

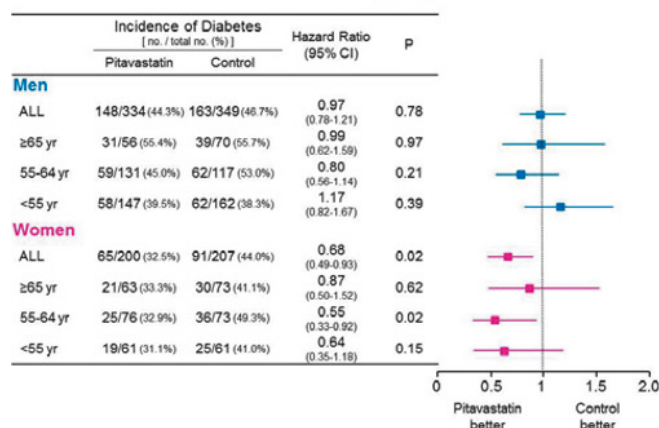
can prevent or delay the development of diabetes, it is helpful for preventing CHD, especially in women. Thus, we conducted a sex-specific analysis in J-PREDICT comparing the effect of pitavastatin (PIT) on the incidence of diabetes in women versus men.

Materials and methods: J-PREDICT study was a prospective randomized, open-label, blinded-endpoint trial evaluating the effect of PIT on the incidence of diabetes ($n=1,269$; 635 Control [lifestyle modification alone], 634 PIT [PIT 1-2 mg + lifestyle modification]) in Japanese subjects with impaired glucose tolerance (IGT). The primary outcome was incidence of DM defined as 2-h plasma glucose of ≥ 200 mg/dl or fasting plasma glucose of ≥ 126 mg/dl measured at least once in 75g OGTT performed every six months. This study was performed based on the full analysis set (total, $n=1090$; men, $n=683$; women, $n=407$).

Results: Women were older than men and lower in body mass index. Women and men had similar levels of glycemic and insulin-related parameters. The incidence of diabetes in women was lower by 32% (HR, 0.68; 95%CI, 0.49-0.93; $p=0.02$) in the PIT group compared with control group, but not significantly different between the group in men (HR, 0.97; 95%CI, 0.78-1.21; $p=0.78$). The analyses using stratified log-lank test and Cox proportional hazard model also brought a positive PIT effect only in women. Trends towards higher incidence of diabetes with increasing age were observed only in men. The beneficial effect of PIT was most in a group of 55 to 64 years in both genders, and only significant in women ($p=0.02$). Thus, incidence of diabetes in women was not associated with age (<55 years: HR, 0.64; 95%CI, 0.35-1.18; 55-64 years: HR, 0.55; 95%CI, 0.33-0.92; ≥ 65 years: HR, 0.87; 95%CI, 0.50-1.52). As for lipid parameters, PIT significantly reduced LDL cholesterol compared with control in men and women, but HDL cholesterol was significantly increased only in men.

Conclusion: PIT reduced the incidence of diabetes in women with IGT. This beneficial effect in women was not associated with age. PIT treatment may be effective even in women with moderate or low risk of CHD.

Effect of Pitavastatin on the Incidence of Diabetes in Men and Women Stratified According to Age



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Can delaying the onset of type 2 diabetes be cost-effective?

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Background and aims: Type 2 diabetes is a 21st century epidemic, but lifestyle and some pharmaceutical interventions can delay its onset in at-risk subjects. We evaluated what effects a delay in diabetes onset by a hypothetical intervention could have on quality-adjusted survival and lifetime complication costs in UK and US healthcare settings, and likely cost-effectiveness at different intervention costs.

Materials and methods: We used data representing the characteristics of 3,058 NAVIGATOR trial subjects at the time they progressed from impaired glucose tolerance to new-onset diabetes, and simulated their remaining life-

time clinical outcomes using the UKPDS Outcomes Model. Multiple simulations examined the impact of a hypothetical intervention that delayed diabetes onset by 1, 3, 5 or 7 years on costs and (quality-adjusted) life expectancy. For simplicity, we assumed that all patients would experience the same period of delay regardless of their characteristics or previous medical history. All future costs and effects were discounted at 3.5% (UK) and 3.0% (US).

Results: Subjects were mean (1SD) age 66.3 (6.9) years, HbA1c 43 (7) mmol/mol, body mass index 31.5 (5.7) kg/m², and 51% were male. The longer diabetes onset was delayed the greater was the increase in life expectancy and quality-adjusted life years (QALYs) and the decrease in the costs of diabetic complications. When diabetes onset was delayed by 7 years, undiscounted life expectancy measured from the simulation baseline increased from 13.8 to 15.4 years; discounted QALYs increased from 7.6 (US \$8.0) to 8.4 (US \$8.8) years, with a corresponding decrease in the discounted costs of complications from £16,741 (US \$140,967) to £16,377 (US \$140,369). Assuming an annual cost for the hypothetical intervention of £1,000 (US \$1,600), the estimated cost per QALY gained when onset was delayed by one year was £7,240 (US \$11,546), falling to £6,909 (US \$10,251) when onset was delayed by 7 years. If the annual cost for the hypothetical intervention was £3,000 (US \$4,800), the estimated cost per QALY gained when onset was delayed by one year was £22,691 (US \$34,870), falling to £21,302 (US \$32,239) when onset was delayed by 7 years.

Conclusion: In our analysis, interventions that can delay the onset of diabetes substantially increased remaining life expectancy and quality-adjusted survival and slightly reduced remaining lifetime complication costs. At annual costs of between £1,000 and £3,000 (US \$1,600 and \$4,800), the hypothetical intervention yielded a cost per QALY gained that remained broadly within the ranges considered cost-effective in the UK and US. Further work is required to explore heterogeneity in the patient population and parameter uncertainty in the modelling. Developing such interventions continues to be a high priority, especially given the continued growth in the global population with type 2 diabetes.

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PS 002 Aetiological epidemiological studies of type 2 diabetes

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Blood glucose levels at baseline and incidence of type 2 diabetes: a prospective cohort study of 0.5 million adults in the China Kadoorie Biobank

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Background and aims: Random blood glucose (RBG) levels may be used to screen for diabetes, however the predictive value of RBG levels within the normoglycemic range on future diabetes risk has not been well studied, particularly among Chinese population.

Materials and methods: The study population includes 496,720 individuals enrolled into the China Kadoorie Biobank between 2004–8 from 10 diverse localities across China without prior physician diagnosed diabetes. At baseline, RBG was measured by venous blood spot test and those with RBG ≥ 11.1 mmol/L and those fasting blood glucose ≥ 7.0 mmol/L were identified as newly detected diabetes (2.8%) and excluded from the current analysis. Data on type 2 diabetes incidence was collected through electronic linkage with mortality and morbidity registries as well as with the national health insurance system. RBG was related to incidence of type 2 diabetes using Cox proportional Hazard models, with adjustment for potential confounders.

Results: The overall mean age of participants ($n=474,423$) was 51 years, mean BMI was 23.6 kg/m², and mean RBG was 5.7 mmol/L. During the 7-year follow-up, 4194 incident cases of type 2 diabetes were identified. A dose-response relationship was observed between baseline RBG and risk of diabetes. Compared to those with RBG < 6.0 mmol/L, those with $6.0 \leq \text{RBG} < 6.9$ mmol/L had a diabetes hazard ratio (HR) 2.8 (95% CI: 2.7–2.9), those with $7.0 \leq \text{RBG} < 7.8$ mmol/L had HR 3.8 (3.5–4.1) and those with RBG ≥ 7.8 (i.e. pre-diabetes) had HR 6.8 (6.3–7.4). The association of RBG with diabetes risk was slightly stronger in women than in men, and in younger than in older people. Stratifying the analyses by hours since last meal (< 2 hours, 2–4 hours, 4–6 hours, or ≥ 6 hours) revealed a stronger association among those who had fasted for a longer period (HR of RBG ≥ 7.8 mmol/L 12.0 [5.3–27.2]).

Conclusion: In this group of adult Chinese, higher RBG levels, even those within the normoglycemic range, constitute an independent risk factor for type 2 diabetes. Such levels should probably be taken into account in identifying people at increased risk for diabetes.

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Stressful life events and the metabolic syndrome: the Hoorn Study

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Background and aims: The amount of psychosocial stress may play a role in the development of the Metabolic Syndrome. The aim of our current study was to evaluate whether in a population-based cohort of older men and women the number of stressful life events is associated with the Metabolic Syndrome incidence, and whether any such relationship is mediated by behavioural factors.

Materials and methods: The association between the number of stressful life events experienced at baseline and Metabolic Syndrome incidence after 6 years of follow up, was assessed in the Hoorn study. Subjects with the Metabolic Syndrome at baseline, defined according to the Adult Treatment Panel III (NCEP), were excluded.

Results: We included 1099 participants (47% male; age 60.1 \pm 7 years). During 6.5 years of follow-up, 238 subjects (21.7%) developed the Metabolic Syndrome. Using logistic regression, a positive association was observed between

Metabolic Syndrome incidence at follow-up and the number of stressful life events [OR 1.11 (1–1.25)]. However, we also observed effect modification for education level (P value interaction, $p=0.01$). In the low-education group, the model adjusted for age and sex showed a significant association between Metabolic Syndrome incidence and the amount of stressful life events [OR 1.20 (1.03–1.39)]. No such significant association was observed in the middle/high-education group [OR 1.05 (0.83–1.24)]. Additionally, a chi-square analysis showed a linear-by-linear association between the number of Metabolic Syndrome abnormalities and the amount of stressful life events ($p<0.01$) in the low educated group. Finally, we observed that smoking, high alcohol intake and low physical activity did not mediate the association between stressful life events and Metabolic Syndrome incidence.

Conclusion: Experiencing more stressful life events is associated with a significantly increased risk for developing metabolic syndrome in an elderly population-based cohort, especially in persons with a low education level.

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Circulating fractalkine levels predict the development of the metabolic syndrome

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Background and aims: The fractalkine/CX3CR1 axis plays an important role in regulating glucose and lipid metabolism. However, the role of fractalkine in metabolic disorders remains to be fully elucidated.

Materials and methods: We selected 887 Chinese (40–65 years old) at baseline, with a subgroup of 459 participants examined again 2 years later. The relationship of serum fractalkine levels with the metabolic syndrome (Mets) and its components was investigated.

Results: At baseline, participants with MetS had a higher fractalkine concentrations than their counterparts without MetS ($P<0.001$). At the 2-year follow-up, participants in the highest quartile of baseline fractalkine exhibited higher values for body mass index, waist circumference, waist-to-hip ratio, body fat percentage, blood pressure, glucose, insulin, total cholesterol, triglycerides (TG), homeostasis model assessment of insulin resistance (HOMA-IR), and lower value for high density lipoprotein-cholesterol (HDL-c) (all $P<0.05$). Among 390 participants without MetS at baseline, 45 developed it at year 2. Even after multiple adjustments for visceral adipose tissue area, HOMA-IR, C-reactive protein (CRP) or TG and HDL-c, baseline fractalkine predicted the development of MetS (OR=7.18, 95%CI: 2.28–18.59).

Conclusion: Circulating fractalkine predicts the development of the MetS independently of central obesity, CRP, insulin resistance and dyslipidemia.

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Influence of serum osteocalcin and liver fat content on glucose metabolism in a middle-aged and elderly Chinese population

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Background and aims: The interaction among adipose tissue, liver and bone has been disclosed. Dysregulation of this network promotes the development of metabolic diseases, including diabetes. We aimed to investigate the relationship of serum osteocalcin (OCN) secreted by osteoblasts, liver fat content (LFC) and glucose metabolism in a middle-aged and elderly Chinese community population.

Materials and methods: A cross-sectional study was performed on 3782 eligible participants from Shanghai Changfeng Community Study. LFC was measured via a newly-established ultrasound quantitative method. One-way ANOVA and multivariate linear regression analysis were carried out to determine the independent and joint association of OCN and LFC with glucose metabolism status.

Results: Decreased serum OCN was associated with higher body weight, waist circumference, diastolic blood pressure, serum triglyceride, fasting and postprandial blood glucose, fasting insulin and insulin resistance level (represented by HOMA-IR) as well as higher LFC (all $P<0.05$). There was a synergistic increase seen in serum fasting blood glucose, postprandial glucose, insulin and HOMA-IR level in individuals with both increased LFC and decreased serum OCN (Figure 1). Multivariate regression analysis showed that both OCN and LFC were independently associated with FBG (std

$\beta=-0.122, P<0.001$ and std $\beta=0.110, P<0.001$), PBG (std $\beta=-0.074, P<0.001$ and std $\beta=0.147, P<0.001$) and HOMA-IR (std $\beta=-0.053, P<0.001$ and std $\beta=0.169, P<0.001$).

Conclusion: Our results suggest that both liver and bone contribute to the development of diabetes and serum OCN and LFC may have a synergistic effect on human glucose metabolism.

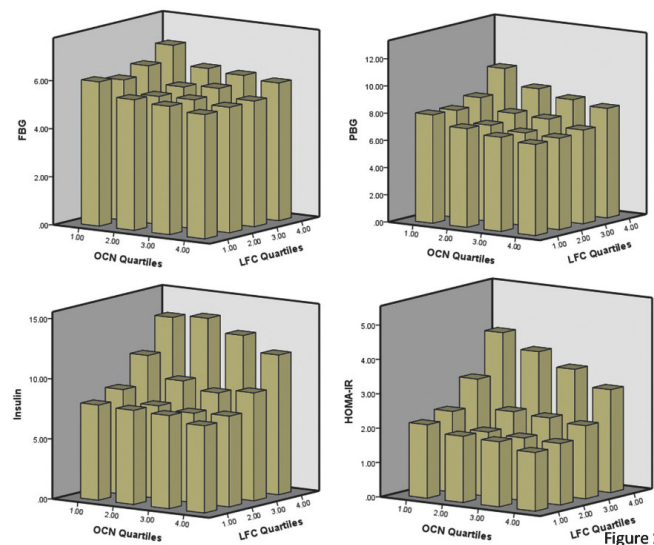


Figure 1

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Cardio-metabolic parameters in non-diabetic patients with chronic kidney disease

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Background and aims: The nature of the relationship of obesity and insulinresistance (IR) with adverse clinical outcomes in chronic kidney disease (CKD) is unclear. The aim of the study was to assess association of cardio-metabolic parameters with estimated glomerular filtration rate (eGFR) in CKD and non-CKD non-diabetic subjects.

Materials and methods: We performed a cross-sectional analysis in a random sample of 1391 non-diabetic subjects, aged 20–79 years, derived from PREDATORR study. CKD was defined as eGFR <60 ml/min/1.73m² or urinary albumin to creatinine ratio ≥ 30 mg/g. The analysed cardio-metabolic parameters were: waist circumference (WC), waist to height ratio (WHtR), body mass index (BMI), body adiposity index (BAI), visceral adiposity index (VAI), glycemia, insulinemia, HOMA-IR, uric acid, lipid profile, systolic and diastolic blood pressure (SBP, DBP).

Results: The prevalence of CKD was 7.5%, majority of CKD patients being in the age group 60–79 years. Frequent cardio-metabolic comorbidities, in subjects with CKD, were dyslipidemia (90%), abdominal obesity (89.4%), obesity (84.6%), hypertension (87.5%), prediabetes (52.9%), hyperuricemia (52.9%), IR (43.3%). Obesity, IR and dyslipidemia were prevalent in the young subjects, while abdominal obesity, prediabetes, hypertension and hyperuricemia were prevalent in elderly people. Among the cardio-metabolic comorbidities associated with presence of CKD found to be statistically significant were obesity, abdominal obesity hyperuricemia, prediabetes, hypertension ($p<0.001$ for all), IR ($p=0.002$) and dyslipidemia ($p=0.006$). Young subjects with CKD had significantly higher WC (99.7 ± 11 vs 88.3 ± 17 ; $p<0.001$), WHtR (0.6 ± 0.08 vs 0.5 ± 0.08 ; $p=0.004$), BMI (34.2 ± 7.2 vs 25.3 ± 5.1 ; $p<0.001$), BAI (35.6 ± 10.5 vs 27.3 ± 5.8 ; $p=0.01$), VAI (2.5 ± 1.7 vs 1.7 ± 1.5 ; $p=0.02$), glycemia (81.9 ± 10 vs 74.6 ± 10.4 ; $p=0.04$), insulinemia (17 ± 9.9 vs 10.3 ± 8.8 ; $p=0.005$), HOMA-IR (3.5 ± 2.4 vs 1.6 ± 1.1 ; $p=0.003$) and uric acid (6.1 ± 1.4 vs 4.7 ± 1.4 ; $p=0.006$), compared to non-CKD young subjects. Elderly CKD subjects had only higher HbA1c (5.7 ± 0.4 vs 5.5 ± 0.3 ; $p<0.001$), insulinemia (12.8 ± 7.4 vs 11 ± 9.1 ; $p=0.001$), HOMA-IR (2.7 ± 2 vs 2.4 ± 2 ; $p=0.003$) and uric acid (6.3 ± 1.7 vs 5.4 ± 2.3 ; $p<0.001$) compared to non-CKD elderly subjects. In CKD subjects

eGFR was negative correlated with age ($r=-0.3$; $p<0.001$), uric acid ($r=-0.3$; $p=0.002$) and positive correlated with WC ($r=0.2$; $p=0.02$), WHtR ($r=0.2$; $p=0.03$), BMI ($r=0.2$; $p=0.01$). Multiple linear regression indicated that age and uric acid were negatively associated with eGFR, while WHtR was positive predictor. In non-CKD subjects a negative correlation occurred between eGFR and age and, also, all cardio-metabolic parameters (WC, WHtR, BMI, VAI, BAI, glycemia, HbA1c, HOMA-IR, triglycerides, total and LDL cholesterol, uric acid, SBP, DBP). Multiple linear regression indicated that only age, uric acid, SBP and HbA1c were negatively associated with eGFR.

Conclusion: This study proposed a synergistic association between cardio-metabolic syndrome components and the presence of CKD. Obesity parameters were associated with CKD in young subjects, while HOMA-IR and uric acid were associated with CKD independently of age. WHtR and uric acid may be important pathogenic factors in non-diabetic CKD patients.

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Associations of sleep duration and sleep efficiency with glucose tolerance and beta cell function in Hong Kong Chinese children and adolescents

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Background and aims: Increasing evidence suggest a role of sleep duration on metabolic dysregulation and dysglycemia in adults. We aimed to examine the associations of sleep duration and sleep efficiency with glucose metabolism in Hong Kong Chinese children and adolescents.

Materials and methods: Children and adolescents of Chinese ethnicity aged 6–18 years were identified from a population-recruited, territory-wide survey. All subjects completed 3-day sleep diary and 3-day actigraphy for the assessments of subjective and objective sleep duration and sleep efficiency. Oral glucose tolerance test (OGTT) and insulin assays were performed. Glucose tolerance, insulin sensitivity, and pancreatic β -cell function were assessed by 2-hour plasma glucose level, Matsuda index (IS_{OGTT}), and insulin secretion-sensitivity index-2 (ISSI-2), respectively. Subjects with moderate-to-severe obstructive sleep apnea (OSA) as determined by an obstructive apnea hypopnea index (OAH) > 5 /hour in an overnight polysomnography were excluded from analyses. Multiple linear regression analyses were performed to explore the associations between sleep and glucose metabolism parameters. Variables with skewed distribution were log-transformed before entering into analyses.

Results: A total of 131 children and adolescents were studied. Among them, 13 had moderate-to-severe OSA, leaving 118 subjects eligible for the present analysis (mean age \pm SD: 13.1 ± 3.3 years; male: 44.9%). After adjustment for gender, Tanner stage, BMI z-score, and OAH, sleep duration and sleep efficiency measured by actigraphy were negatively associated with log-transformed 2-hour plasma glucose levels ($\beta \pm$ se: -0.042 ± 0.022 , $p = 0.054$ and -0.009 ± 0.004 , $p = 0.033$, respectively) and positively associated with log-transformed ISSI-2 ($\beta \pm$ se: 0.102 ± 0.049 , $p = 0.038$ and 0.023 ± 0.009 , $p = 0.014$, respectively). There were no significant associations of log-transformed IS_{OGTT} ($p > 0.20$) with sleep duration and sleep efficiency as measured by actigraphy. Moreover, no associations were found between subjective sleep duration and sleep efficiency as measured by sleep diary and all glucose metabolism parameters as mentioned above ($p > 0.20$) (Table 1).

Conclusion: Short sleep duration and low sleep efficiency as measured by actigraphy are independent risk factors for worse glucose tolerance and β -cell function in children and adolescents.

Table 1: The associations of sleep duration and sleep efficiency measured by 3-day actigraphy and 3-day sleep diary with glucose/insulin metabolic parameters

Outcomes	Predictors	β	Standard error	P value [¶]
Log-transformed 2-h glucose levels	Sleep duration by actigraphy	-0.042	0.022	0.054
	Sleep efficiency by actigraphy	-0.009	0.004	0.033*
	Sleep duration by sleep diary	0.01	0.018	0.570
	Sleep efficiency by sleep diary	0.134	0.173	0.437
Log-transformed ISSI-2	Sleep duration by actigraphy	0.102	0.049	0.038*
	Sleep efficiency by actigraphy	0.023	0.009	0.014*
	Sleep duration by sleep diary	-0.009	0.039	0.820
	Sleep efficiency by sleep diary	-0.295	0.384	0.444
Log-transformed IS_{OGTT}	Sleep duration by actigraphy	0.063	0.069	0.363
	Sleep efficiency by actigraphy	0.002	0.013	0.893
	Sleep duration by sleep diary	-0.028	0.055	0.617
	Sleep efficiency by sleep diary	-0.539	0.537	0.318

[¶] Multiple linear regression analyses adjusted for gender, Tanner stage, BMI z-score, obstructive apnea hypopnea index; * $P < 0.05$.

IS_{OGTT} : Matsuda index; ISSI-2: insulin secretion-sensitivity index-2.

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Glycated albumin is a useful indicator for screening impending diabetes and predicting beta cell dysfunction in the pre-diabetic condition

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Background and aims: Pre-diabetes is known as a pre-clinical stage of increased risk for overt diabetes mellitus (DM) and cardiovascular disease. Because glycated albumin (GA) has been suggested to have more potential for assessing insulin secretory dysfunction and glycemic fluctuation than HbA1c, we studied the clinical significance of GA in this stage.

Materials and methods: We enrolled the 1379 anti-diabetic drug naïve subjects in retrospective, multi-center, cross-sectional manner. According to the 75-g OGTT, the subjects were classified as normal glucose tolerance (NGT), isolated IFG (i-IFG), isolated IGT (i-IGT), combined glucose intolerance (CGI) and DM subgroup. We analyzed clinical characteristics of these 5 groups including GA, insulin sensitivity (HOMA2%S), and insulin secretion (HOMA2%B) index.

Results: Mean GA was 11.6 ± 1.4 , 12.3 ± 1.8 , 12.3 ± 1.9 , 13.0 ± 1.9 , 18.8 ± 7.9 in NGT ($n=295$, 21.4%), i-IFG ($n=257$, 18.6%), i-IGT ($n=103$, 7.4%), CGI ($n=257$, 18.6%), and DM ($n=466$, 34%) subgroup. After adjusting covariates, adjusted mean of GA was 12.2 ± 0.1 , 12.2 ± 0.2 , 13.1 ± 0.1 in i-IFG, i-IGT, and CGI subgroup ($p<0.001$), and significantly higher in CGI group by post-hoc analysis. Adjusted mean of HbA1c was also significant, but not distinguished differences among these subgroups. Moreover, correlation coefficient between HOMA2%B and GA ($r=-0.393$, $p<0.001$), and HOMA2%S and GA ($r=0.258$, $p<0.001$) was significantly higher than correlation with HbA1c. And these results were consistent after adjusting covariates.

Conclusion: We suggest that GA could be a better indicator for screening impending diabetes and assessing beta cell dysfunction in the subjects of pre-diabetic period.

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A population approach to modelling the oral glucose tolerance test in normal and impaired glucose tolerant states

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Background and aims: The conventional approach to analyzing oral glucose tolerance testing (OGTT) data requires model identification in each individual separately (standard two stage), ignoring knowledge about the population as a whole. However, the OGTT is sparsely sampled and individual estimates are often not resolvable from the available data.

Materials and methods: We applied a population approach, nonlinear mixed effects modeling, to plasma glucose, insulin and C-peptide data obtained from a 120-minute OGTT undertaken by 106 subjects forming five groups with varying glucose tolerance. This method provides estimates of population means, variances and covariances of model parameters, empirical Bayes estimates of individual parameter values and measures of intra-individual (within-subject) and inter-individual (between-subject) variability. The latest version of the oral glucose minimal model was used to evaluate insulin sensitivity and a combined model approach was used to assess β -cell secretion. These models allowed for the reconstruction of insulin secretion and glucose absorption profiles and gave population indexes of insulin sensitivity ($S_I = 6.51 \pm 1.20 \times 10^{-4} \text{ min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$), fractional hepatic extraction of insulin ($F = 0.522 \pm 0.291$) and fractional insulin clearance ($k_I = 0.258 \pm 0.151 \text{ min}^{-1}$).

Results: Individuals with type 2 diabetes (T2DM) had significantly higher HOMA-IR, HbA1c and triglyceride levels compared to subjects with normal glucose tolerance (NGT). They also had increased fasting plasma insulin levels, significantly reduced insulinogenic index from 0 to 20 minutes after oral glucose, and increased fasting glucagon levels compared to NGT subjects. All had similar fasting plasma biochemical values and were without medical histories of gastroparesis, kidney disease or microvascular disease. The population model, including data from all groups of individuals, was implemented in NONMEM. When empirical post hoc Bayes estimates obtained from the population approach were used to determine individually predicted values, these points collapsed to the line of unity, showing no systematic deviations from the observed data. NGT subjects were found to have the highest median values for S_I , as well as the most variability. Mean estimates and standard errors for S_I ($104 \text{ min}^{-1} \cdot \mu\text{U}^{-1} \cdot \text{ml}$) in the different groups were: Group 1 (normal fasting and 2-hr plasma glucose; 17.7 ± 1.8), Group 2 (normal fasting / impaired 2-hr plasma glucose; 9.95 ± 2.0), Group 3 (impaired fasting and 2-hr plasma glucose; 5.67 ± 1.0), Group 4 (normal fasting / diabetic 2-hr plasma glucose; 3.82 ± 1.1) and Group 5 (diabetic fasting and 2-hr plasma glucose; 9.39 ± 3.8). Individual insulin secretion profiles were also reconstructed from empirical Bayes estimates.

Conclusion: Whereas the traditional approach to parameter estimation failed to recover estimates in more than one third of the population, the population approach provided individual estimates in all subjects. Examination of the empirical Bayes estimates showed that individual parameter estimates were able to differentiate well between individuals at glucose tolerant states ranging from euglycemia to overt type 2 diabetes. Our findings suggest population analysis is a powerful tool to obtain accurate assessments of indexes of insulin sensitivity and β -cell function from the OGTT, especially in epidemiological studies with large numbers of sparsely sampled subjects.

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Family history is not associated with lifestyle, clinical, or anthropometric factors in newly diagnosed type 2 diabetes mellitus patients: the DD2 studyE. Svensson¹, K. Berensci¹, S. Sander¹, A. Mor¹, J. Rungby², J.S. Nielsen³, S. Friberg⁴, I. Brandslund⁵, J.S. Christiansen⁶, A. Vaag⁷, H. Beck-Nielsen³, H.T. Sørensen¹, R.W. Thomsen¹;

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Background and aims: A positive family history of type 2 diabetes mellitus (T2D) increases the risk for developing T2D approximately two-fold, likely due to both genetic and lifestyle factors. It is unknown how having a family history of T2D is related to demographic, clinical-, lifestyle and anthropometric factors at T2D debut, thus we aimed to examine this in a cross-sectional study.

Materials and methods: All participants in the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort responded to a questionnaire, in which detailed family history of diabetes (grandparents, parents, siblings and children) was assessed. We examined the prevalence and relative risk, and corresponding 95% confidence interval (CI) of having one first-degree relative vs no first-degree relative with history of T2D according to different demographic, lifestyle, clinical and anthropometric factors at T2D debut.

Results: Of 2,718 T2D patients, 1,191 (44%) had one or more first-degree family relatives with diabetes, including 377 (14%) with two or more first-degree relatives. Of the patients with positive family history, 20% had affected mothers, while 17% had affected fathers. Among the newly diagnosed T2D patients, a family history of T2D was to a lesser extent seen in men compared with women (adjusted relative risk (aRR) 0.84, 95% CI 0.78–0.90). Individuals aged less than 40 years at their T2D debut were more likely to have a family history of diabetes as compared with patients aged more than or equal to 60 years at T2D debut (aRR 1.34, 95% CI 0.94–1.91). In contrast, family history of T2D was evenly distributed according to presence or absence of central obesity, large weight gain since age 20, physical activity, alcohol consumption and Charlson comorbidity score.

Conclusion: Almost half (44%) of newly diagnosed T2D patients have a first degree family relative with diabetes. Our results confirm that T2D heredity is stronger on the maternal side. Having a family history of diabetes is thus associated with female gender and younger age at T2D debut, whereas family history does not appear to be associated with presence of comorbidity or specific lifestyle-, clinical- and anthropometric factors at debut.

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Determinants of skin autofluorescence in the general population and in type 2 diabetesB.H.R. Wolffenbuttel¹, R. Graaff¹, H.L. Lutgers¹, K. Eny², A.D. Paterson², S.N. Slagter¹, J.V. van Vliet-Ostapchouk¹, M.M. van der Klauw¹;

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Background and aims: Skin autofluorescence (SAF) is a non-invasive marker of advanced glycation end products and is associated with long-term diabetes complications and cardiovascular morbidity and death. SAF increases with age, and is higher among individuals with diabetes.

Materials and methods: We assessed the determinants of SAF in people with and without type 2 diabetes (T2DM) in the LifeLines Cohort study, a random population-based sample of inhabitants of three Northern provinces of the Netherlands. For this cross-sectional analysis, we included subjects 18–90 years of age, who had both genetic data available and SAF measurement collected between January 2008 and March 2011. We excluded subjects who were known to have type 1 diabetes and those with serum creatinine $>140 \text{ mcmol/l}$, leaving 9025 individuals (of whom $n=314$, 3.5% with T2DM) for analysis. Skin autofluorescence (SAF) was measured non-invasively with the AGE Reader.

Results: Mean (\pm SD) age was 49 (\pm 11) yrs for the non-diabetic participants and 59 (\pm 11) yrs for the T2DM group, while mean SAF was 2.04 (\pm 0.44) arbitrary units (AU) vs 2.45 \pm 0.59 AU. Linear regression showed that age, sex, body mass index, HbA1c, glomerular filtration rate (GFR), smoking, and a genetic polymorphism of N-acetyltransferase 2 (NAT2) significantly influenced SAF in the general population (all $p < 0.001$) and type 2 diabetic subjects (all $p < 0.009$). In addition, total cholesterol was an independent predictor of SAF in diabetes. Finally, the effect size of sex, HbA1c, smoking and NAT2 were larger in subjects with T2DM than in the general population (Table).

Conclusion: We conclude that SAF is influenced by several clinically significant parameters, and some of them bear a more significant influence in T2DM. These factors should be taken into account when using SAF as a predictor of cardiovascular and diabetes-related complications. The large effect of smoking in T2DM supports increasing our efforts to promote smoking cessation in those individuals.

	Non-diabetic n=8711			Type 2 diabetes n=314		
	B	SE	P	B	SE	P
(Constant)	0.825	0.075	6.0×10^{-28}	1.049	0.296	4.6×10^{-4}
Age	0.020	0.001	4.8×10^{-299}	0.021	0.003	3.6×10^{-9}
Sex	-0.059	0.008	8.6×10^{-13}	-0.134	0.051	8.7×10^{-3}
Body mass index	0.009	0.001	4.0×10^{-11}	0.019	0.007	4.8×10^{-3}
HbA1c	0.050	0.014	2.2×10^{-4}	0.073	0.022	1.3×10^{-3}
Glomerular filtration rate	-0.001	0.000	5.7×10^{-8}	-0.003	0.001	2.4×10^{-3}
Smoking	0.202	0.009	3.0×10^{-100}	0.453	0.066	2.9×10^{-11}
rs4921914_C (NAT2)	-0.115	0.007	4.9×10^{-61}	-0.184	0.045	5.6×10^{-5}
Total cholesterol	-0.007	0.004	0.092	-0.069	0.021	1.2×10^{-3}

PS 003 Type 1 diabetes: epidemiology

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Environmental pollutants and the risk of type 1 diabetes

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Background and aims: Environmental pollutants have been associated with immune aberrancies, but their role as a risk factor of type 1 diabetes is not known. We explored the association of the plasma levels of environmental pollutants and later development of type 1 diabetes associated beta-cell autoantibodies in children at genetic risk of type 1 diabetes.

Materials and methods: We analyzed the plasma levels of 13 persistent organic pollutants (POP) and 13 perfluorinated compounds (PFC) in a series of cord blood samples and plasma samples taken at the age of 12 months from children at genetic risk of type 1 diabetes who participated in the FINDIA pilot study and were monitored for the appearance of type 1 diabetes associated autoantibodies. Plasma samples were pretreated with dispersive solid phase extraction. The PFCs and POPs were extracted with methanolic ammonium acetate and dichloromethane:hexane (1:4), respectively. The sample volume used for the analysis ranged from 25 μ L to 200 μ L. Instrumental analysis was performed with the LCMS/MS (PFAs) and GC-MS/MS (POPs).

Results: The levels of 13 POPs or 13 PFCs did not differ in cord blood samples or plasma samples taken at the age of 12 months between the children who developed autoantibodies and children who remained autoantibody negative during the follow-up over the first 6 years of life. No association of environmental pollutants with the development of clinical type 1 diabetes was observed. Increased levels of both POPs and PFCs were associated with breast-feeding.

Conclusion: Our results do not suggest that early exposure to POPs or PFCs during pregnancy or early infancy is a risk factor for the later development of beta-cell autoimmunity and type 1 diabetes.

Clinical Trial Registration Number: NCT01055080

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Enteroviral infection in human type 1 diabetes: correlative evidence from multiple tissue sources in nPOD samples

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Background and aims: Considerable evidence has accumulated to support the hypothesis that type 1 diabetes (T1D) is associated with an enteroviral infection. Much of this depends on the analysis of viral infection in blood samples but definitive proof that this is also associated with the presence of enterovirus within the organs of affected individuals is still lacking. The purpose of this study was to undertake the first fully coordinated analysis of multiple tissues available within the JDRF's network of pancreatic organ donors with diabetes (nPOD) collection. These tissues were analysed using different methods, in multiple collaborating laboratories, to assess the correlation of potential indices of enteroviral infection.

Materials and methods: Tissue samples (pancreas/ spleen/ PBMCs) from controls, T1D and autoantibody positive (AAb+) cases were prepared by the nPOD Pathology Core and distributed in a blinded manner to each laboratory. Samples were immunostained for enteroviral capsid protein VP1 and class I MHC [using IHC in FFPE sections and IF in frozen sections] and probed by *in situ* hybridisation for enteroviral genome. Enterovirus sequence analysis was attempted in extracts prepared from frozen spleen and PBMCs in a selection of the cases.

Results: Expression of enteroviral capsid protein, VP1, and hyper-expression of class I MHC were detected consistently in the islets of Langerhans of patients with T1D while VP1 was also detected in the spleens. Immunopositivity was remarkably concordant between the different laboratories, despite

the different methodologies employed. Tissues from a total of 101 cases were then examined and scored for several indices of viral infection including VP1 immunopositivity, class I MHC hyperexpression, positive *in situ* hybridisation signals and the presence of viral sequences. Among 28 controls, a total of 54 indices of viral infection were assessed independently and 7 were reported as positive (13%). In 52 type 1 diabetes cases (including those with residual insulin-containing islets and those without) 99 indices were scored and 51 were positive (51.5%). Among 21 autoantibody positive cases, 43 indices were assessed and 16 were positive (37.2%). PCR amplification and nucleic acid sequencing analysis of RNA extracted from cultured spleen cells or PBMCs revealed the presence of several enterovirus serotypes and the positivity correlated with VP1 staining in sections of tissue from the same samples.

Conclusion: The results support the conclusion that enteroviral infection occurs at much higher frequency in T1D pancreas than in controls. Virus can be found both in blood samples and in other tissues (e.g. pancreas, spleen). Similar evidence of persisting virus was also found in AAb+ cases, supporting the hypothesis that enteroviruses may play a role early in the development of T1D.

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Increased antibodies against other autoimmune diseases in first degree relatives of patients with type 1 diabetes

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Background and aims: It is well known that T1D is associated with other autoimmune diseases. The aim of this study was to compare the prevalence of various auto-antibodies in first-degree relatives of patients with T1D and healthy individuals with negative family history of diabetes.

Materials and methods: The group studied consisted of 90 relatives and 60 healthy individuals. Serum concentrations of antibodies to anti-21-hydroxylase (21-OH-Abs), anti-gastric parietal cell antibodies (GPC-Abs), anti-thyroglobulin antibodies (TG-Abs), anti-thyroid peroxidase antibodies (TPO-Abs) and anti-TSH receptor antibodies (TSHR-Abs) were measured by commercial radioimmunoassay.

Results: Positive antibodies against pancreatic islet antigens were found in 34.4% of the relatives (IAA in 23.3%, GADA in 16.7% and IA-2A in 2.2%) and in none of the controls. Other antibodies (mainly TPO-Abs, TSHR-Abs and GPC-Abs) were detected in 40% of all relatives and in 93.5% of these with positive anti-islet antibodies. Median levels of 21-OH-Abs, GPC-Abs, TPO-Abs and TSHR-Abs were significantly higher in the relatives, in particular these with positive anti-islet antibodies, as compared with the group of relatives with no anti-islet antibodies and the controls. A positive correlation between IAA and TPO-Abs levels was noted in the whole group of relatives, as well as in a subgroup with anti-islet antibodies ($r=0.549$, $p<0.05$ and $r=0.567$, $p<0.05$, respectively).

Conclusion: Our results demonstrated for the first time significantly higher prevalence of anti-thyroid antibodies and anti-gastric parietal cell antibodies in the first-degree relatives of T1D patients, in particular in these with positive anti-islet antibodies. The finding suggest that these subjects may be at higher risk of developing not only type 1 diabetes, but also other autoimmune disorders and should be routinely screen especially for autoimmune thyroid disease and auto-immune gastritis.

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Prevalence of diabetes autoantibodies in Chinese adult patients with type 1 diabetes mellitus and its relation with beta cell function

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Background and aims: There are limited data on the frequency of beta-cell autoantibodies in Chinese adult patients with type 1 diabetes mellitus (T1DM). Our study aimed to explore the prevalence of diabetes autoantibodies (DAs) and its relation with beta-cell function in a cohort of adult patients with T1DM in Guangdong Province, China.

Materials and methods: Data were derived from Guangdong T1DM Translational Medicine Study. A total of 1070 patients with T1DM (488 males and 582 females) who were ≥ 18 years of age and had DAs measurements available were included in this analyses. The median age was 31.7 years old and duration was 4.14 years. Autoantibodies (GADA, IA2A and ZnT8A) were measured by radioligand assay confirmed by Diabetes Antibody Standardization Program. Fasting C-peptide (FCP) and postprandial C-peptide 2 hours (PCP2h) were measured centrally by standard methods.

Results: 548(51.2%) subjects were positive for at least one antibody and 47(4.4%) subjects were positive for three antibodies. Among the subjects with single positive antibody, 324(89.3%), 37(10.2%) and 2(0.5%) participants were positive for GADA, IA-2A and ZnT8A, respectively. Detailed data were shown in Figure 1. DA-positive participants were younger at diagnosis (median: 25.8 vs. 27.2 years), more likely to be female, with lower BMI at diagnosis (median: 18.34 vs. 19.49 kg/m²), lower FCP levels (median: 0.12 vs. 0.17 ng/ml) and PCP2h levels (median: 0.13 vs. 0.22 ng/ml) and higher HbA_{1c} levels (median: 8.8% vs. 8.4%), compared with those who were DA negative ($P<0.05$ for each). A correlation was found between the number of positive antibodies and disease duration ($r_s=0.064$, $P=0.04$) and FCP concentration ($r_s=-0.149$, $P=0.00$). As the increasing of disease duration, a tendency could be also observed that the rate of decline of FCP could be faster in the DA-positive group (Figure 2).

Conclusion: Our data showed the prevalence of diabetes autoantibodies in Chinese adult patients with T1DM was lower than the countries with high incidence of T1DM. The characteristics of DA-positive participants could suggest a more aggressive clinical status. There might be a more progressive decline in beta-cell function on account of diabetes autoantibodies.

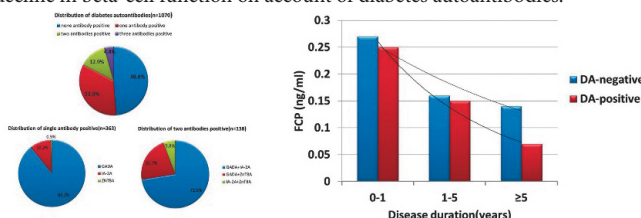


Figure 1: Prevalence of diabetes autoantibodies

Figure 2: FCP concentration by disease duration

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Relationship between glycaemic variability, functional beta cell mass and glucose disposal rate in (pre)type 1 diabetes

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Background and aims: Novel beta cell therapies in (pre)type 1 diabetes (T1D) aim to preserve or restore a functional beta cell mass (FBM) that avoids large glycemic excursions. In order to define therapeutic target populations, we examine which functional reserve is needed to curb glycemic variability (GV). As a first step we investigated cross-sectionally the correlation between GV (assessed by continuous glucose monitoring [CGM] and self-monitoring-of-blood-glucose [SMBG]) and measures of FBM and of insulin resistance.

Materials and methods: The study was conducted in 9 recent-onset T1D patients (13-36 years; $n=3$ in remission defined as insulin dose <0.5 U.kg⁻¹.day⁻¹) and in 21 persistently autoantibody-positive first-degree relatives (autoAb⁺ FDR) with overall 50% 5-year disease risk (12-41 years; $n=4$ with impaired glucose tolerance [IGT] at baseline). All underwent CGM (iPro2, Medtronic) for 5 days followed by hyperglycemic clamp test (10.0 mmol/l). FBM was calculated as AUC C-peptide release between min120-150, and glucose disposal rate (M) as average glucose infusion rate between min120-150 minus a space correction. We derived 90 different GV parameters from CGM measurements using the GlyVarT 1.0 program (Medtronic) and 4 GV parameters from SMBG data (Contour Link, Bayer).

Results: Overall, GV parameters (CGM: interquartile range_{day}, SD_{day} and %glycemia_{day} >7.7 mmol/l; SMBG: SD_{day} and range_{day}) were inversely correlated with FBM ($r=-0.6$ to -0.7 , $p\leq 0.001$). All patients except 1 in remission and 4/21 FDR had a FBM \leq percentile 10 (P10) of healthy controls; 2 of these 4 FDR developed T1D within 9 months. Among the 17 FDR with FBM >P10, 2 exhibited elevated GV parameters. A stronger correlation was found between GV parameters and M values ($r=-0.8$, $p<0.001$). Most patients (except 2 in remission) had M values \leq P10 of healthy controls, vs 0/21 FDR. Of the 7/21 FDR with M \leq P33 of controls, all had ($n=4$ including 1 with preT1D) or developed IGT ($n=2$) or T1D ($n=1$) within 4–10 months vs only 1/14 FDR with higher M; their GV parameters were also higher ($p=0.007$ to 0.053 vs FDR with M >P33). In autoAb⁺ FDR, M \leq P33 of controls detected presence or development of dysglycemia within 1 year with 88% sensitivity and 93% specificity. Especially when considering multiple parameters, CGM was more sensitive than SMBG in detecting individuals who later developed IGT or T1D.

Conclusion: In autoAb⁺ FDR low glucose disposal rate, also reflecting insulin resistance, outperforms FBM in diagnosing or predicting dysglycemia and is closely related to elevated CGM parameters. GV could provide non-invasive outcome measures in clinical trials.

Supported by: JDRF (USA), EU FP-7 (Brussels), FWO (Brussels)

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Baseline and five year treatment characteristics of adult onset type 1 diabetes

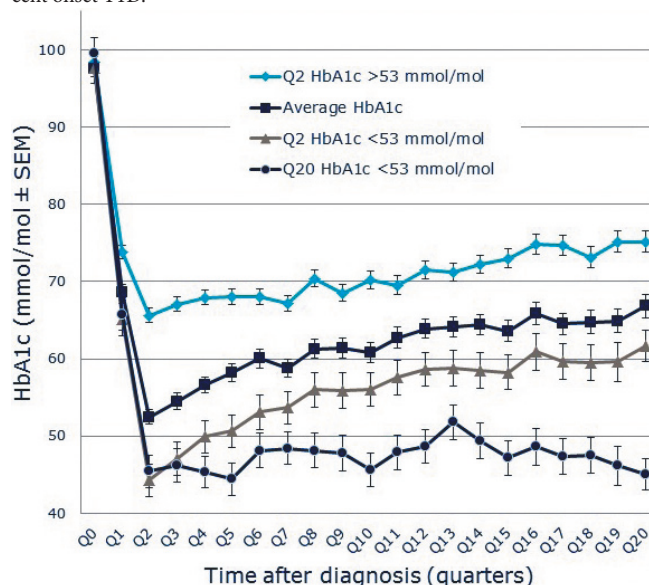
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Background and aims: Much less is known about adult onset type 1 diabetes (AOT1D) than childhood or adolescent onset T1D. We sought to characterize the initial five years of treatment of AOT1D.

Materials and methods: Subjects with AOT1D between 2001 and 2012 were identified in the electronic medical records of our diabetes center. Baseline demographics and diabetes related parameters for up to five years after onset were registered. Patients with latent autoimmune diabetes of the adult (LADA) were not included. Data are shown as mean \pm standard deviation. Non-parametric tests were used throughout and a p-value <0.05 was considered statistically significant.

Results: 487 patients with AOT1D were identified (men/women 61/39%), age at onset 35.2 ± 13.6 years. 383 patients were followed for as long as possible for up to five years for this study. Patients leaving the center, some of which returned, were also accounted for. GAD antibody status was available in 335 subjects (standard onset test since 2005-08-01). 274 patients were GAD positive and 61 GAD negative (82/18%). IFCC HbA1c at onset was 97 ± 30 mmol/mol ($n=487$). At the five year follow-up HbA1c had decreased from 97 ± 30 to 69 ± 18 mmol/mol (Kruskal Wallis [KW] $p<0.0001$; Figure 1). Patients with an HbA1c <53 mmol/mol after 5 years (17%; 31 out of 178 subjects with 5 year data: 45 ± 4 vs. 71 ± 16 mmol/mol; $p<0.0001$) were already showing a significantly lower HbA1c by the second quarter after diagnosis (45 ± 7 vs. 53 ± 12 mmol/mol; $p<0.005$). There was no difference in HbA1c between these groups at diagnosis (100 ± 20 vs. 97 ± 31 mmol/mol; ns), nor concerning other diabetes related variables. Apart from HbA1c, the only other five-year outcome that was different between the groups was a lower total insulin dose in the group <53 mmol/mol (36 ± 22 vs. 52 ± 23 IU; $p=0.0001$). The likelihood of having a five year HbA1c <53 mmol/mol was four times higher in subjects who reached an HbA1c <53 mmol/mol within the first six months of treatment compared to those who did not (odds ratio 4.2 [1.4–13.0] 95%CI; $p=0.01$). Over the five years, there were significant but modest increases in blood pressure, HDL-cholesterol, and BMI, and a significant decrease in triglycerides (KW $p<0.0001$ for all), with no change in LDL-cholesterol; most changes occurring during the first two years. By contrast, there was a gradual increase in total insulin dose (KW $p<0.0001$). Only 14 patients were transferred from multiple daily injections to continuous subcutaneous insulin injection during the five first years of treatment.

Conclusion: Tight glycemic control in subjects with AOT1D can be maintained over time. Already within half a year from the onset/diagnosis it may be possible to predict the long term sustainability of the glycemic treatment by looking at the HbA1c level. The lack of other predictive variables for long term prognosis, as well as the lack of indicators for the need of alternative strategies, is striking and motivates further research into this large group of patients, including comparisons with patients with childhood- and adolescent onset T1D.



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After diagnosis diabetes research support system-2 (ADDRESS-2): clinical presentation of type 1 diabetes in the beginning of the 21st century in a multi-ethnic UK cohort

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Background and aims: Type 1 diabetes (T1D) most commonly presents with the classical triad of polyuria, polydipsia and weight loss but duration and pattern of symptoms may vary. The reported frequency of diabetic ketoacidosis (DKA) at presentation of T1D is 10 to 80% That has changed little over the last decades in the developing and the developed world. The purpose of our study was to determine the frequency of DKA at presentation of T1D, in a multi-ethnic UK cohort of patients and study their clinical characteristics. **Materials and methods:** The ADDRESS-2 cohort comprises people with incident T1D, and includes clinical and demographic features and, in many, islet-specific antibodies. From 01.09.11 to 31.12.13, children over 5 and adults under 60 years with T1D were recruited to ADDRESS-2 from 134 UK recruiting sites, within 6 months of diagnosis.

Results: 1,440 patients were recruited (1,264 white; 65% male; 55% children; mean age 20.1y, SD 12.95; median age 15y, IQR 10–28). At presentation 96.6% described osmotic symptoms, 85.8% weight loss and 84.7% fatigue. Subjects presented with none (0.6%), one (5.6%), two (20.2%) or all three symptoms (73.8%). 43.7% presented with DKA. Symptom duration ranged from under 2 weeks to over 12 months (median 3w, IQR 2–6). There was no association between symptom type and age, gender or ethnicity. Symptom duration increased significantly with increasing age ($p<0.01$), female gender ($p<0.01$) and Black ethnicity ($p=0.02$). There was no significant difference in age between those presenting with or without DKA (DKA: median 15.5y, IQR 11–28; non-DKA: median 15y, IQR 10–28). Gender and ethnicity were not significantly different between those presenting with DKA and those without. 92% of those presenting with DKA suffered weight loss, 90% fatigue and 97% osmotic symptoms. Weight loss and fatigue were significantly more frequent with DKA ($p<0.01$) but osmotic symptoms occurred at comparable frequency ($p=0.11$). Increasing number of symptoms was significantly associated with DKA presentation ($p<0.01$). Mean symptom duration in those

with DKA (5.1 weeks, SD 6.2) and those without (6.5 weeks, SD 18.8) was comparable ($p=0.42$). Positive autoantibody (AAb) status was not associated with symptom type, number or duration or DKA at presentation. GAD AAb titre had no effect on frequency of presentation with DKA. 8.2% of those presenting with DKA had at least one other autoimmune condition versus 4.5% of those with no DKA ($p<0.01$). There was no overall significant difference in weight, height, BMI or birth weight between those with DKA or not. Family history of diabetes did not correlate with symptoms but paternal diabetes was significantly more frequent in the non-DKA population ($p=0.01$).

Conclusion: Our study offers important insight into clinical and anthropometric characteristics of patients presenting with T1D in a modern multi-Ethnic European country. The duration of symptoms is typically short and increases with age, female gender and Black ethnicity. Weight loss, fatigue and co-existing autoimmune disease are commoner in those with DKA. The frequency of DKA at presentation of T1D remains very high, suggesting that T1D remains a public-health issue.

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Serum bilirubin is inversely related to both HbA_{1c} and complications in people with type 1 diabetes

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Background and aims: There is evidence of an inverse correlation between HbA_{1c} and serum bilirubin in people with type 2 diabetes and in healthy populations in the Far East. We have investigated this further in a UK population with type 1 diabetes, and also whether there is any association between bilirubin and microvascular complications.

Materials and methods: Data were extracted from the Nottingham University Hospitals diabetes register and pathology databases and the Nottingham retinopathy screening database. Of 2067 adults registered with type 1 diabetes, we excluded those with renal failure, pregnancy or a recent diagnosis of diabetes.

Results: 1595 people (77% of those eligible) had HbA_{1c} and serum bilirubin levels within a 15 month window. Mean (standard deviation) age was 45 (15) years with duration of diabetes 22 (13) years. There was an inverse relationship between HbA_{1c} and bilirubin ($R = -0.15$, $P<0.001$) which persisted in a stepwise linear regression model including also sex, age and duration of diabetes. Mean HbA_{1c} in the group with the lowest quintile of bilirubin was 76 (19) mmol/mol compared with 66 (16) mmol/mol in the highest quintile. The results were not significantly altered by excluding those with a serum bilirubin above the reference range. The group with the lowest quintile of bilirubin concentration had a higher prevalence of microalbuminuria (27.7 vs 14.2%, $P<0.001$) and of retinopathy (29.0 vs 17.7%, $P=0.011$), compared with the remainder of the cohort.

Conclusion: There is a significant inverse correlation between HbA_{1c} and bilirubin in people with type 1 diabetes in a UK population. Microvascular complications were more prevalent in those with a low bilirubin concentration, suggesting the inverse association with HbA_{1c} is not solely a biochemical artefact.

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LDL-cholesterol is not a good marker of cardiovascular risk in type 1 diabetes: observational study in 30,778 patients: a report from the national diabetes register in Sweden

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Background and aims: Patients with type 1 diabetes (T1D) are at high risk of cardiovascular disease (CVD). High LDL-cholesterol (LDL) is an important risk factor in general population and in type 2 diabetes. Less is known in T1D. The aim of this study was to assess LDL and total-cholesterol to HDL-cholesterol ratio (Chol/HDL) as predictors of CVD in T1D and to evaluate the risk at different levels of LDL.

Materials and methods: 30,778 T1D patients, age 18–79 years, were included 2003–2006 and followed for mean 6.8 years. Mean age 46 years, diabetes duration 21 years, HbA_{1c} 8% (NGSP), LDL 2.7 mmol/L and Chol/HDL 3.2. 10% had a history of CVD. 26.6% ($n = 8172$) were treated with lipid-lowering

medication, they were older, had longer diabetes duration and 24% had previous CVD.

Results: There were 13.8 CVD events/1000 person years (py) in patients without and 51.7 events/1000 py in patients with lipid-lowering medication. Cox regression analyses were performed with LDL and Chol/HDL as predictors and fatal/nonfatal CVD as outcome, adjusted for other CVD risk factors. Adjusted hazard ratios (HR) per 1 mmol/L increase in LDL for CVD were 1.03 (95% CI 0.99–1.08) in all, 1.05 (1.00–1.14) in those without and 1.04 (0.98–1.1) in those with treatment. All HR were non-significant. Adjusted HR per 1 unit increase in Chol/HDL for CVD were 1.08 (1.05–1.12) in all, 1.11 (1.05–1.16) in patients without and 1.08 (1.03–1.13) in patients with lipid-lowering medication. All p -values < 0.01 . We also assessed risk of CVD with octiles of LDL, octile 4 as reference (LDL 2.5–2.7). Compared to reference only patients without lipid-lowering treatment, in the highest octile (LDL > 3.6 mmol/L) had significant higher risk HR 1.25 (1.02–1.52).

Conclusion: In our study LDL was not a significant predictor of CVD in T1D apart from in patients in the highest octile not treated with lipid-lowering medication. The ratio of Chol/HDL was a significant predictor for CVD in all groups. The ratio of chol/HDL seems to be a more reliable marker for risk.

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Women from South Asia, Middle East and Africa at increased risk of postpartum weight retention and type 2 diabetes:

a multi-ethnic population based cohort study in Oslo, Norway

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Background and aims: Pregnancy has been considered a critical period for development of overweight and its complications including type 2 diabetes, both in the mother and through diverse mechanisms including epigenetic modifications, also in her child. Immigrant women of Asian and African origin living in Europe are at higher risk for obesity, type 2 diabetes and cardiovascular diseases than the native European population. Women with gestational weight gain above recommended levels from American Institute of Medicine are at increased risk of postpartum weight retention and thereby of later obesity and related co-morbidities. The aim of the study was to explore ethnic differences in postpartum weight retention three months postpartum in a population-based, multi-ethnic study of pregnant women.

Materials and methods: A multi-ethnic population based cohort study from Oslo, Norway of 823 healthy pregnant women, 59 % with ethnic minority background, included from 2008–2010. A total of 642 (78% of 823) were followed till three months postpartum. A multiple linear regression analyses were performed to model the relationship between postpartum weight retention and ethnicity.

Results: Unadjusted mean postpartum weight retention was 2.3 kg (95% confidence interval 1.7–2.9) for women from Western Europe and ranged from 3.7–6.3 kg among ethnic minority groups. The proportion of women in the highest quintile (postpartum weight retention 8.5–24.4 kg) differed by ethnicity; 12% among Western European, 9% among East Asians, 25% among South Asians, 28% among Middle Eastern, 29% among East European and 41% among African women ($p < 0.001$ for all versus Western Europeans, except for East Asians and Eastern Europeans). Postpartum weight retention as percentage increase of pre-pregnant body weight was 3.3% among Western Europeans, 6.5% among East Asians, 7.6% among Middle Eastern, 8.0% among Eastern Europeans, 8.4% among South Asians and 9.3% among women from Africa. After adjustments for age, gestational weight gain, parity and education, women from South Asia retained 2.8 kg (95% confidence interval 1.9–3.6), Middle East 2.0 kg (95% confidence interval 1.0–3.0) and women from Africa 4.4 kg (95% confidence interval 3.1–5.8) more than Western Europeans ($p < 0.001$).

Conclusion: Significantly more women from South Asia, Middle East and Africa had postpartum weight retention in the highest quintile compared to Western European women.

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The 'obesity paradox' and mortality in adults with type 2 diabetes: explained by collider biases

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Background and aims: Among adults with type 2 diabetes (T2D), being overweight or obese near the time of diagnosis is associated with reduced risk of mortality compared with normal-weight patients - the 'obesity paradox'. We tested the hypothesis that the strengthening of the negative association

between smoking and BMI induced by the conditioning on the disease state, T2D, explains the obesity paradox.

Materials and methods: We developed the logic of collider bias as it pertains to the association between smoking and body mass index (BMI) in 10761 patients with T2D from the Salford Integrated Records database (Manchester, UK). We related peri-diagnosis BMI (within a year of diagnosis) and mortality using flexible parametric survival analyses with age (35 to 85 years) as the timescale, adjusting for sex and stratified by smoking, with BMI 25 to <30 kg/m² as a referent group.

Results: There were 1247 deaths during a mean period of 8.57 years of follow-up. A U-shaped association was observed across BMI categories (18.5 to 25.9, 25.0 to 29.9 [reference], 30.0 to 35.4, 35.0 to 39.9, and ≥ 40.0) for all-cause mortality (hazard ratio plus 95% confidence interval [CI], 1.22 (1.05–1.42); 1.00; 0.97 (0.84–1.13); 1.33 (1.09–1.62); 2.02 (1.59–2.56) respectively). The U-shaped relationship was retained in ever-smokers, but in never smokers, increased risk of mortality was only seen in Obese II–III groups (2.43 CI 1.68–3.50). The U-shaped relation was noted in younger but not older 10-year age bands. Associations remain after excluding patients who died or were censored within 2-years of diabetes diagnosis, suggesting reverse causality was not a major confounder.

Conclusion: We confirmed that, among adult patients with incident T2D, being normal weight or obese II/III is associated with higher mortality compared with overweight and obese I categories, but these observations were explained by collider biases due to associations of age and smoking with mortality in the diseased population. Weight loss interventions to reduce mortality in patients with T2D merits future research.

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Impact of visceral fat on skeletal muscle mass in a prospective cohort study: the Korean Sarcopenic Obesity Study (KSOS)

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Background and aims: To investigate whether visceral obesity promotes a decrease in skeletal muscle mass and vice versa.

Materials and methods: We observed changes in anthropometric and body composition data during a follow-up period of 27.6 ± 2.8 months in 379 Korean men and women (mean age 51.9 ± 14.6 years) from the Korean Sarcopenic Obesity Study (KSOS), an ongoing observational prospective cohort study.

Results: Appendicular skeletal muscle mass (ASM) and total skeletal muscle mass were calculated using dual-energy X-ray absorptiometry, and visceral fat area (VFA) was measured using computed tomography at baseline and follow-up examination along with the various kinds of confounding or possible mediating factors. Both ASM and total skeletal muscle mass significantly decreased, whereas trunk and total fat mass increased in both men and women despite no significant change in weight and body mass index. Correlation analysis adjusting for age and gender revealed that baseline VFA was negatively correlated with changes in ASM ($P = 0.001$). However, baseline ASM was not significantly associated with changes in VFA ($P = 0.135$). In particular, women with visceral obesity at baseline had a greater decrease in ASM than those without visceral obesity ($P = 0.001$). In multiple linear regression analysis, baseline VFA was an independent negative predictor of the changes in ASM after adjusting for confounding factors including age, gender, life style parameters, insulin resistance, high sensitivity C-reactive protein and vitamin D levels ($P = 0.001$), whereas the association between baseline ASM and changes in VFA was not statistically significant ($P = 0.142$).

Conclusion: This longitudinal study showed that visceral obesity was associated with future loss of skeletal muscle mass in Korean adults. These results may provide novel insight into sarcopenic obesity in an aging society.

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Visceral fat is associated with hyperglycaemia after renal transplantationM. von Düring¹, T. Jenssen^{1,2}, J. Bollerslev³, K. Godang³, A. Aasberg⁴, A. Hartmann¹;¹Department of Organ Transplantation, Section of Nephrology, University of Oslo, Rikshospitalet, ²Institute of Clinical Medicine, Faculty of Health Science, University of Tromsø, ³Section of Specialized Endocrinology, ⁴Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Rikshospitalet, Norway.

Background and aims: Hyperglycaemia and new onset diabetes after transplantation (NODAT) are common complications in renal transplant recipients and are associated with impaired long-term survival. Studies in the non-transplant population suggest that visceral fat facilitates development of type 2 diabetes. The role of visceral fat for development of NODAT is not known. The aim in this study was to elucidate this relationship between visceral fat content and hyperglycaemia in renal transplant patients.

Materials and methods: We studied 159 renal transplant patients without a prior diagnosis of diabetes. All of them underwent oral glucose-tolerance tests (OGTTs) in a stable phase 10 weeks after transplantation. Visceral fat content was analyzed by a newly validated software (CoreScan) applied after total body composition DXA-scans using Lunar Prodigy, software version 14.10.

Results: The amount of visceral fat (median 1.0 kg, interquartile range = IQR 0.4 - 1.9 kg) was highest in patients with NODAT (median 2.2 kg, IQR 1.2-2.7). There was a significant difference in the amount of visceral fat between the categories of glucose tolerance; NODAT, impaired glucose tolerance (median 1.2 kg), impaired fasting glucose (median 1.0 kg) and normal glucose tolerance (median 0.8 kg) (Kruskal-Wallis ANOVA, $p = 0.003$). The percentage visceral fat of the total fat mass was 97% higher in patients with NODAT (median 7.7%, IQR 6.0-8.6%) compared with NGT patients (median 3.9%, IQR 2.0-5.8%) ($p < 0.001$). Percentage visceral fat of total fat mass was also a better predictor of both fasting (FPG, $R^2 = 0.116$, $p < 0.001$) and 2-hour plasma glucose (2hPG, $R^2 = 0.082$, $p < 0.001$), compared to total body fat per se or BMI in a multiple regression analysis.

Conclusion: Visceral fat is adversely associated with glucose metabolism in renal transplant recipients. Whether reduction of visceral fat mass may prevent development of NODAT needs further studies.

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Increased risk for diabetes development in non-diabetic Korean subjects with hypertriglyceridaemic waist phenotype

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Background and aims: Hypertriglyceridemic waist (HTGW) phenotype is a simple and inexpensive screening parameter to identify people at increased risk for cardiovascular disease. We evaluated whether the HTGW phenotype predicts diabetes in Korean urban adults.

Materials and methods: 2,900 non-diabetic subjects (mean age 44.3 years) including 2078 male (71.7%) and 822 female (28.3%), who did annual medical check-up in a university hospital for four consecutive years were recruited. The subjects were divided into four groups according to the baseline serum triglyceride (TG) and waist circumference (WC); normal WC-normal TG (NWNT), normal WC-high TG (NWHT), high WC-normal TG (HWNT), high WC-high TG (HWHT). High serum TG was defined as ≥ 150 mg/dL and high WC was defined as ≥ 90 cm for men and ≥ 85 cm for women. New cases of diabetes were determined according to the self-questionnaire of the participants and fasting plasma ≥ 126 mg/dL. Cox proportional hazard model analysis was used to assess the cumulative incidence of diabetes according to baseline HTGW phenotypes.

Results: 101 (3.5%) new diabetes cases were diagnosed during four years of follow-up period. The subjects in HWHT group had the highest incidence of diabetes (8.3%) compared with the NWNT group (2.2%). The adjusted hazard ratio (aHR) for developing diabetes in the presence of HWHT phenotype at baseline was 4.11 (95% confidence interval [CI] = 2.40-7.06) after adjusting for age, and 2.44 (95% CI = 1.38-4.33) after adjusting for age, sex, total cholesterol and systolic pressure, when NWNT group was considered as the reference group.

Conclusion: This study demonstrated that HWHT showed the highest risk for diabetes development during four years of follow-up period. Recognizing HWHT type would be useful to identify individuals at high-risk of diabetes and, which is of great significance in reducing the incidence of diabetes among Korean urban adults.

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Metabolically healthy obesity, presence or absence of fatty liver, and risk of type 2 diabetes in Japanese individualsY. Heianza^{1,2}, Y. Arase², K. Fujihara¹, S. Hsieh², K. Saito^{1,2}, O. Hanyu¹, S. Kodama^{1,2}, S. Hara², H. Sone^{1,2};¹Department of Internal Medicine, Niigata University Faculty of Medicine,²Health Management Center, Toranomon Hospital, Tokyo, Japan.

Background and aims: Whether a “metabolically healthy obese (MHO) phenotype” that does not include typical obesity-related metabolic abnormalities is really a benign or malignant state has not been elucidated in terms of predicting the progression to type 2 diabetes. We investigated whether the MHO phenotype was associated with an increased risk of the development of diabetes. If so, we aimed to determine what factors could explain this finding.

Materials and methods: Studied were 8090 Japanese individuals without diabetes (diabetes indicated by the ADA criteria). Metabolic health status was assessed by common clinical markers: blood pressure, triglycerides, HDL-cholesterol, and fasting glucose concentrations. Cut-off value for obesity (O) or normal weight (NW) was a BMI of 25.0 kg/m^2 . Participants were categorized at the baseline examination into 4 phenotypes: 1) metabolically healthy and normal weight (MHNW), 2) metabolically healthy and overweight or obese (MHO), 3) metabolically abnormal and normal weight (MANW), or 4) metabolically abnormal and overweight or obese (MAO).

Results: The 5-year incidence rate of diabetes was 1.2% ($n=58/4749$) in MHNW individuals, 2.8% ($n=20/719$) in MHO individuals, 6.0% ($n=102/1709$) in MANW individuals, and 10.3% ($n=94/913$) in MAO individuals. Although the MHO individuals had no or 1 metabolic factor, 47.8% had ultrasonographic fatty liver (FL). The MHO group had a significantly increased risk of diabetes compared to the MHNW group (multivariate-adjusted OR, 2.23 (95% CI 1.33, 3.75)), but this risk was attenuated after adjustment for FL. We then assessed the combined effect of obese phenotypes and FL on the development of diabetes. MHNW/non-FL group had the lowest incidence rate of diabetes (0.9%), and MHO/non-FL group also had a similarly low incidence rate of diabetes (1.1%). On the other hand, the MHO/FL group had an elevated incidence rate (4.7%). Incidence rate of diabetes was markedly high at 8.5% in the MANW/FL group and 12.6% in the MAO/FL group. Compared to the MHNW/non-FL group, the risk of diabetes in the MHO/non-FL group was not significantly elevated (OR 1.01 (0.35, 2.88)). However, the MHO/FL and MHNW/FL groups had similarly elevated risks of diabetes (OR 4.09 (95% CI 2.20, 7.60) and 3.16 (1.78, 5.62), respectively). A prediction model with age, sex, and obese phenotypes had an area under the ROC curve (AUCROC) of 0.754 for the development of diabetes. The AUCROC was slightly but significantly ($p < 0.001$) improved when we added the assessment of FL into the model, with an area under the ROC curve of 0.778. The net reclassification improvement was 8.8% (95% CI 1.7% to 15.8%) by introducing the assessment of fatty liver in the prediction model.

Conclusion: Our results showed that individuals with MHO as defined in this study had a significantly higher risk of the development of diabetes compared to MHNW individuals without consideration of the presence of ectopic fat in the liver. However, at baseline almost half of MHO individuals had an accumulation of fat in liver as assessed by ultrasonography, and this observation partially explained the increased risk of diabetes among the MHO individuals. The presence of FL should be evaluated to assess whether an individual was actually in a metabolically benign state in predicting the development of diabetes.

Supported by: JSPS

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Presence and severity of obstructive sleep apnoea is independently associated with glycometabolic abnormalities in obese non-diabetic subjects

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Background and aims: Obstructive sleep apnea (OSA) is a common underdiagnosed condition in the obese population, and has been associated with worse glycemic control in individuals with type 2 diabetes. The relationship between OSA and glycometabolic parameters was investigated in obese non-diabetic individuals.

Materials and methods: Ninety-one obese subjects (57% male, mean age 45.3 ± 12 yrs, mean BMI 42.1 ± 9 kg/m²) underwent polysomnography and a 2-h oral glucose tolerance test (OGTT).

Results: OSA was identified in 64% of subjects (73% in male, 41% in female, $p=0.032$ χ^2). Obese subjects with OSA showed higher A1c (5.8% vs 5.5%, $p=0.009$), plasma glucose at 120 min during OGTT (PPG120) (133 mg/dl vs 102 mg/dl, $p=0.001$), triglyceride (140 mg/dl vs 117 mg/dl, $p=0.045$) and uric acid (5.8 mg/dl vs 4.9 mg/dl, $p=0.035$) levels than obese subjects without OSA. A1c levels and PPG120 were found to be significantly correlated with raised apnea-hypopnea index (AHI) ($p=0.007$ and $p=0.004$, respectively), oxygen desaturation index ($p=0.002$ and $p=0.04$, respectively), and percent of sleep time with oxyhaemoglobin saturation at <90% (ST90) ($p=0.002$ and $p=0.004$, respectively). Furthermore, increasing quartiles of ST90 and AHI were associated with increasing levels of A1c and PPG120 ($p=0.015$ and $p=0.003$, respectively, for ST90; and $p=0.035$ and $p=0.014$, respectively, for AHI). Multiple regression analysis showed that ST90 was the strongest independent determinant of A1c, after controlling for sex, age, BMI, waist circumference, CRP, HOMA-IR ($\beta=0.442$, $p=0.007$). Similarly, AHI persisted as an independent determinant of A1c ($\beta=0.414$, $p=0.005$). Both ST90 and AHI persisted as determinants of PPG120, albeit not significantly ($\beta=0.377$, $p=0.095$; and $\beta=0.358$, $p=0.119$, respectively).

Conclusion: OSA acts as an independent factor exacerbating the metabolic risk attributed to obesity. Recognition and treatment of OSA may decrease the progression toward type 2 diabetes in obese subjects.

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Retrospective analysis of fatty liver markers for incident diabetes: comparison between people with lower alcohol intake and higher alcohol intake

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Background and aims: Although fatty liver is a risk factor for incident diabetes, markers of fatty liver for incident diabetes have yet to be sufficiently investigated. We retrospectively analyzed fatty liver markers including serum cholinesterase (ChE) for incident diabetes and compared them between people with lower alcohol intake and higher alcohol intake in accordance with cut-off point for non-alcoholic fatty liver disease (ethanol intake 140g/week).

Materials and methods: Study periods I and II were defined as January 2007 to May 2009 and June 2009 to December 2011, respectively. A total of 2084 people (1389 men, 695 women; mean age: 46 years) who underwent an annual medical check-up including blood sampling after an overnight fast in Periods I and II were recruited. They were accumulated as representatives of the diurnal regional population. We excluded people positive for hepatitis B virus antigen and hepatitis C virus antibody as well as those with diabetes in Period I. Others were divided into lower alcohol intake (LAI) group (ethanol intake ≤ 140 g/week, $n=1515$) or higher alcohol intake group (HAI) group (ethanol intake > 140 g/week, $n=448$). In this study, diabetes was diagnosed by prescription of antidiabetic agents, fasting plasma glucose (FPG) ≥ 6.8 mmol/l, HbA1c (NGSP) $\geq 6.5\%$ and/or a past history of diabetes in those not yet treated with antidiabetic agents. Fatty Liver Ultrasonography scores (FLUS) were also assigned as follows: 2 points, subjects with moderate or severe fatty liver; 1 point, those with mild fatty liver; 0 points, those with normal liver. Regression coefficient (B) was calculated using logistic regression analysis.

Results: The mean observation period was 2.23 years, with 27 LAI subjects and 15 HAI subjects with newly developed diabetes from Periods I to II. The remaining 1488 LAI subjects and 433 HAI subjects were nondiabetic. In logistic regression analysis, alanine aminotransferase (ALT) was independently and significantly ($B=0.025$, $p=0.017$) associated with incident diabetes as well as age ($B=0.065$, $p=0.008$), BMI ($B=0.264$, $p=0.050$), waist circumference (WC) ($B=-0.120$, $p=0.036$), fasting plasma glucose (FPG) ($B=0.116$, $p<0.001$) in Period I in the LAI group. Systolic and diastolic BP, Gamma-GTP, ChE, FLUS, triglyceride and HDL cholesterol were not associated. In receiver operating characteristic (ROC) analysis, the specific cut-off point in ALT in Period I for detecting incident diabetes was 24U/l (AUC 0.73, $p<0.001$, sensitivity 70%, specificity 70%) in the LAI group. In the HAI group, ChE was independently and significantly ($B=0.013$, $p=0.009$) associated with incident diabetes as well as HDL cholesterol ($B=-0.069$, $p=0.046$) and FPG ($B=0.315$, $p<0.001$) in Period I. ALT, age, BMI, WC, systolic and diastolic BP, Gamma-GTP, FLUS and triglyceride were not associated. In ROC analysis, the specific cut-off point in ChE in Period I for incident diabetes was 372U/l (AUC 0.72, $p<0.005$, sensitivity 78.6%, specificity 64.8%) in the HAI group.

Conclusion: Although FPG was a powerful predictor for incident diabetes, ALT and ChE also independently affected incident diabetes in LAI group and HAI group, respectively. The ALT and ChE would be useful fatty liver markers for incident diabetes in non-alcoholic fatty liver disease and alcoholic fatty liver disease, respectively.

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PS 005 Descriptive epidemiology of diabetes

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One normal range for HbA_{1c} in every age? The effect of aging on HbA_{1c} in the people without diabetes

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Background and aims: Goals for HbA_{1c} in National Guidelines and the cut-off value for diagnosis of diabetes are not adjusted for age yet. The Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001–2004 showed a 0.6% increase of HbA_{1c} for non diabetic people over the age of 70 compared to people below the age of 40 years. We evaluated this question in a German population.

Materials and methods: We analyzed the data from 10163 visits of 3861 patients without diabetes (age 44.7y; 18–93y; 72.6% women; BMI 28.0±6.5 kg/m²) which had parallel HbA_{1c} and blood glucose measurements and information about drug treatment. The HbA_{1c} values were divided in 3 age groups (<40y [n=3583]; ≥40 < 70y [n=5454]; ≥70y [n=1126]). Patients with gestational diabetes mellitus, documented use of systemic glucocorticoids or HbA_{1c} ≥6.5% were excluded. A glucose tolerance test was not performed. Data were drawn from the electronic patients record (EMIL[®]) of our university outpatient department for endocrinology and metabolic disorders between 10/1992 and 01/2014. HbA_{1c} was DCCT adjusted (mean normal range of healthy people 5.05%).

Results: The mean HbA_{1c} (95. percentile) for the groups of age was: <40years 5.0±0.36% (5.6%), ≥40 < 70 years 5.3±0.38% (5.9%), ≥70 years 5.4±0.37% (6.0%). The differences between <40 years vs. 40–70 years, <40 y vs. >70 y (p<0.001) and 40–70 y vs. >70 y (p<0.001) were significant. The mean blood glucose (non fasting) was <40y 4.5±0.6; 5.2±1.1 mmol/l; ≥40 < 70y 5.0±0.74; 5.3±1.0 mmol/l and ≥70y 5.1±0.85; 5.5±1.1 mmol/l. The differences < 40 vs. 40–70y (p<0.001) und >70y (p<0.001); and 40–70y vs. >70y (p<0.001) were also significant.

Conclusion: HbA_{1c} increases significantly with the age of people without diabetes. The use of different cut-off values for the diagnosis of diabetes according to age should be considered. Also HbA_{1c} goals for old people with diabetes should consider the age dependend increase of HbA_{1c}.

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The association of smoking cessation with HbA_{1c} control of diabetes mellitus: a THIN database study

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Background and aims: Smoking increases the risk of developing type 2 diabetes mellitus. However several large observational studies have found during the first 3–5 years following smoking cessation the risk of developing diabetes is significantly greater than in continuing smokers; an association largely explained by weight gain. This increase in risk is temporary and after 10–12 years the risk is equivalent to never-smokers. There is also preliminary evidence from small cohort studies, in those who already have diabetes that control deteriorates during the first year after smoking cessation before it improves. Our objective was to examine whether an association between smoking cessation and diabetes control exists in a large representative sample of the UK population. If deterioration in diabetes control around the time of smoking cessation does occur, this may have implications for enhanced diabetic care at that time.

Materials and methods: A retrospective cohort study (01/01/2005 – 31/12/2010) was assembled using The Health Improvement Network (THIN) database. Inclusion criteria were: patients aged over 18, registered with their practice for at least one year on 01/01/2005, diagnosed with type 2 diabetes mellitus and whose last recorded smoking status prior to 01/01/2005 was current smoker. An adjusted multilevel regression model was developed to investigate the association between change in HbA_{1c} and stopping smoking.

Results: There were 10,692 adults with type 2 diabetes who were current smokers as at 01/01/2005. Of these, 3,131 (29%) quit smoking and remained abstinent for one year or longer. After adjustment for potential confounders, patients who quit smoking had an average increase in HbA_{1c} after quitting of 2.3mmol/l (95% CI 1.91 to 2.77, p<0.001). HbA_{1c} did decrease as abstinence continued although the modelled trajectory showed this did not return to the levels seen in continuing smokers until 3 years after quitting. This increase in HbA_{1c} was not mediated by weight change after quitting smoking.

Conclusion: Smoking cessation is associated with deterioration in glycaemic control in people with type 2 diabetes that persists for three years and appears not to be caused by weight gain. The rise in HbA_{1c} is minor for each patient and clinician but will substantially increase microvascular complications in the whole population, which could be prevented by prompt action to improve glycaemic control on cessation. While we used an algorithm to help minimise the inaccuracies of recording smoking status within the THIN database and adjusted for missing data using imputation, our data is nonetheless limited by the accuracy of the information available within the THIN database. However, it provides the largest study so far on this phenomenon and confirms for the first time preliminary evidence of an association between quitting and worsening glycaemic control. It highlights the need for proactive review of glycaemic control and the need for prompt adjustment of medication in people with diabetes following smoking cessation.

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How useful is HbA_{1c} for diagnosis of diabetes in acute medical admissions?

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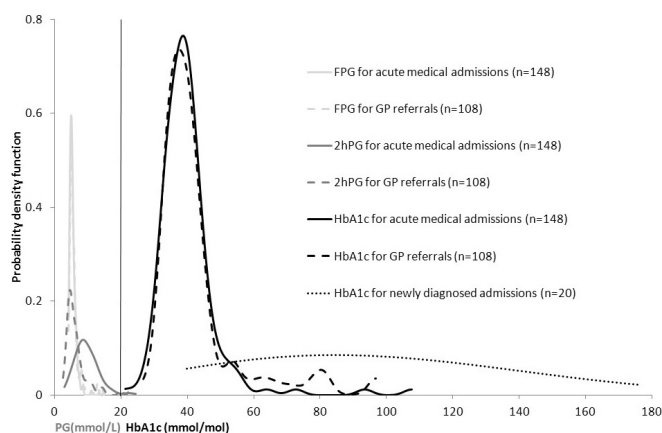
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Background and aims: The detection of hyperglycaemia is important in acutely ill patients who may present with either undiagnosed diabetes, symptoms or complications of diabetes, or stress hyperglycaemia; all indicating worse outcomes.

Materials and methods: Over a 2 year period, 2061 (14%) patients were admitted to hospital by one consultant physician out of a total of 14432 patients. OGTT was performed in 390 (19%) patients during their admission because of suspected symptoms or complications of diabetes. IFCC-calibrated HbA_{1c} was measured by Menarini HA8160 IE HPLC in 148 (38%) of these patients and also 22 (1%) patients diagnosed with diabetes at admission.

Results: The acutely ill patients diagnosed with diabetes immediately were white Caucasian, >18 years old, aged (median IQ range) 36(26 to 61) years, 55% male with admission plasma glucose (PG mmol/L) 19.3(11.8 to 27.4). Fasting PG on OGTT in those with possible symptoms or complications of diabetes, aged 70(59 to 79) years, 63% male with admission PG 6.4(5.6 to 7.4), was 5.2(4.8 to 5.7). This was similar to fasting PG on OGTT of 5.2(4.8 to 5.9) in 108 white Caucasian, primary care patients, aged 54(46 to 61) years, who were at increased diabetes risk, p=0.65. As expected, 2hPG at 9.0(7.3 to 11.4) was higher in acutely ill patients than GP patients, 5.5(4.4 to 7.5), p<0.001. HbA_{1c} (%/mmol/mol) was highest in those diagnosed immediately on admission at 10.2(7.4 to 13.3)/88(57 to 122), p<0.001, and similar in the other groups of patients i.e. 5.7(5.3 to 6.0)/39(34 to 42) in admissions and 5.7(5.4 to 6.1)/39(36 to 43) in GP patients, p=1.00. At OGTT, 15/148 (10%) were diagnosed with diabetes on HbA_{1c} with 14/15 (93%) confirmed on OGTT. More patients, 48/148 (32%), were diagnosed with diabetes on OGTT than HbA_{1c}.

Conclusion: HbA_{1c} could play a diagnostic role in acute medicine as outlined in the ADA 2014 Clinical Practice Recommendations which are based on expert opinion and for which more evidence is requested. Those with very high HbA_{1c} will possibly have been diabetic for some time before admission.



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Achievement of individualised HbA_{1c} treatment targets in patients with type 2 diabetes and comorbid hypertension in a real world setting: results of the DIALOGUE registry

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Background and aims: The recent EASD consensus statement has re-focused on individualized HbA_{1c} Treatment targets in the care of patients with type-2 diabetes mellitus. There are, however, neither clinical practice data on actually pursued treatment targets nor on patient characteristics associated with either strict or loose treatment targets.

Materials and methods: DIALOGUE is a prospective, observational, multi-center registry focusing on treatment targets and their achievement in clinical practice. Physicians were asked at baseline on HbA_{1c} treatment targets pursued and compared to actual HbA_{1c} values achieved at 6 months.

Results: A total of 8,636 patients were included. For 3,371 of these (39.0%) an HbA_{1c} target of ≤6.5% (strict group), for 3,647 (42.2%) a target of >6.5 to ≤7.0% (medium) and for 1,618 (18.7%) a target of >7.0 to ≤7.5% (loose) was pursued. Patients in the strict target group were younger, had lower fasting and postprandial blood glucose values, shorter diabetes duration and less co-morbid disease at baseline than the other groups (table). At the 6 months follow-up the mean (±SD) HbA_{1c} achieved in the strict group was 6.8±0.9% with 25% of patients exceeding 7.1%. Corresponding values for the medium and loose target group were 7.2±0.9 (25% >7.6%) and 7.7±1.2 (25% >8.3%), respectively (p<0.0001). There was a higher rate of long-acting insulin use (15.7%) in the loose target group than in the strict and medium group (8.2 vs. 14.5%) (p<0.0001).

Conclusion: The data illustrate a differentiated selection of HbA_{1c} treatment targets in patients with type-2 diabetes in Germany. Patients with strict targets typically are younger, have lower blood glucose values and less comorbidity, while loose targets are sought in those with existing complications.

Baseline†	HbA _{1c} ≤6.5%‡	>6.5 to ≤7.0%‡	>7.5 to ≤7.5%‡	§
Age (median, IQR)‡	64 (55-73)‡	67 (59-74)‡	66 (58-75)‡	<0.0001‡
Female-sex (%)‡	46.2‡	45.5‡	44.4‡	0.47‡
FBG (mg/dl) (median, IQR)‡	7.3 (6.2-8.7)‡	8.3 (7.0-9.8)‡	8.9 (7.5-11.2)‡	<0.0001‡
PPBG (mg/dl) (median, IQR)‡	9.6 (8.2-11.3)‡	10.7 (9.2-12.8)‡	11.6 (9.9-14.0)‡	<0.0001‡
Diabetes duration (months) (median, IQR)‡	55.7 (24.8-100.6)‡	75.1 (35.7-122.4)‡	82.3 (40.3-131.8)‡	<0.0001‡
CAD (%)‡	23.9‡	23.0‡	26.0‡	0.06‡
Stroke-/TIA (%)‡	5.3‡	6.8‡	6.7‡	<0.05‡
Heart failure (%)‡	11.6‡	13.4‡	17.0‡	<0.0001‡
PAD (%)‡	5.0‡	7.4‡	9.1‡	<0.0001‡

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Achievement of personalised HbA_{1c} targets in patients with type 2 diabetes from the RIACE cohort

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Background and aims: Though the HbA_{1c} goal has been set at 7%, the use of personalized targets is currently recommended by several guidelines. This study was aimed at assessing the percentage of subjects with type 2 diabetes from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study who reach individualized HbA_{1c} goals.

Materials and methods: The RIACE cohort consists of 15,773 patients, consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. Exclusion criteria were dialysis or renal transplantation. HbA_{1c} was measured by HPLC using DCCT-aligned methods. Starting from a baseline HbA_{1c} goal of <6.5%, the target has been arbitrarily increased by 0.5% according to the presence of each of the following criteria: age >70 years; disease duration >10 years; advanced complications; and co-morbidities. As a consequence, 5 HbA_{1c} targets were identified ranging from <6.5% to >8.5%.

Results: Mean HbA_{1c} was 7.55±1.51% (median 7.30%, IQR 6.51-8.28%). The overall prevalence of subjects with HbA_{1c} <7.0% was 40.9% (n=6,453), higher in men than in women (43.2% vs. 37.9%; p<0.0001). The 7.0% goal was reached by 71.0% of patients not taking any medication, 42.1% of those on oral hypoglycaemic agents (OHA), 17.4% of those on OHA+insulin and 24.6% of those on insulin alone (p<0.0001), with a similar trend between genders. In the RIACE cohort, 5,512 subjects (34.9%) were >70 years old, 7,931 (50.3%) had diabetes duration >10 years, 4,819 (30.6%) had ≥1 advanced complication and 2,803 (17.8%), had ≥1 severe co-morbidity. Moreover, 4,110 (G0, 26.1%), 4,914 (G1, 31.2%), 4,444 (G2, 28.2%), 1,957 (G3, 12.4%), and 348 (G4, 2.2%) met 0, 1, 2, 3, and 4 of the above criteria, respectively. The prevalence of subjects meeting personalized goals were: G0 (goal <6.5%) 34.3%; G1 (<7.0%) 41.8%; G2 (<7.5%) 50.2%; G3 (<8.0%) 59.9%; and G4 (<8.5%) 73.8% (p<0.0001); in men: G0 36.1%, G1 43.5%, G2 51.5%, G3 63.5%; and G4 77.5% (p<0.0001); in women G0 32.0%, G1 39.5%, G2 48.3%, G3 55.1%; and G4 68.7% (p<0.0001). The overall prevalence of subjects meeting personalized targets was 45.2% (47.2% in men vs. 42.8% in women, p<0.0001). After excluding subjects with HbA_{1c} <6.5% from G2-G4, the percentage of those on-target was era 37.6% (38.9% in men vs. 36.1% in women, p<0.0001).

Conclusion: Personalizing HbA_{1c} goals increases the prevalence of subjects on-target, especially among those with more advanced disease. However, more than 50% of patients, and particularly 2/3 of those with short-duration, uncomplicated disease, do not meet the recommended HbA_{1c} targets.

Clinical Trial Registration Number: NCT00715481

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Determinants of glycaemic control: a nationwide longitudinal study of 131935 newly diagnosed cases of type 2 diabetes

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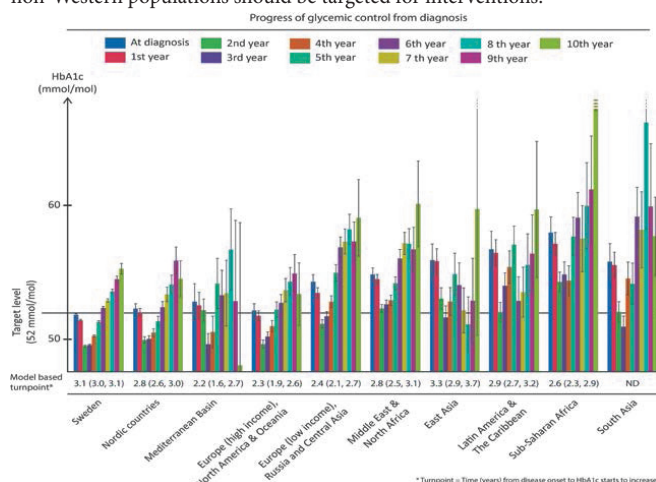
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Background and aims: Studies on glycaemic (HbA_{1c}) control have been compromised by small samples, short follow up, lack of data or unequal access to health care. We used the Swedish National Diabetes Register (2002-2011) to include 131935 newly diagnosed (within one year) cases of type 2 diabetes. We studied glycaemic control and the effect of known and suspected determinants of glycaemic control. Due to knowledge gaps, emphasis was on ethnicity, income and education.

Materials and methods: We assessed sex, age, BMI, duration of diabetes, education, income, ethnicity, physical activity, glucose-lowering treatment, previous cardiovascular disease, risk factors and statin treatment. We report (1) baseline characteristics; (2) progress of glycemic control from disease onset to end of follow up (up to 10 years); (3) linear mixed models to determine the effect of the above mentioned covariates on HbA1c (mmol/mol) and time duration until HbA1c started to increase ('turn point'); (4) logistic regression was used to study the probability of achieving glycemic control (<53 mmol/mol) the 2nd year. Sensitivity analyses were performed.

Results: Phenotype at diagnosis varied markedly by ethnicity; e.g age differed up to 16 years (High-income Europe versus Sub-Saharan Africa) and BMI up to 4 kg/m² (High-income Europe versus South Asia). Glycemic control varied strikingly by ethnicity (figure 1) and socioeconomic status. Non-Western ethnicities displayed considerably higher HbA1c levels throughout follow-up. Overall, HbA1c levels started to increase after 2.9 (95% CI 2.8, 3.0) years but non-Western groups experienced the turn point up to 1 year earlier (figure 1). Target level of HbA1c was out of reach for non-Western ethnicities. Kaplan-Meier graphs revealed that this was not due to late initiation of pharmacologic treatment; paradoxically, non-Western ethnicities received pharmacologic treatment earlier. Linear models enhanced the ethnic differences in HbA1c. Interestingly, we found a strong interaction between ethnicity and type of glucose-lowering treatment (not detailed here). Sub-Saharan Africa, South & East Asia and the Middle East & North Africa displayed poorest control; generally 3–6 mmol/mol higher than high-income Europe. Higher income, but not education, was associated with lower HbA1c levels. Statin use was consistently associated with lower HbA1c (range 0.2–1.0 mmol/mol, depending on model). Logistic regression showed that income, education and, particularly, ethnicity are strongly associated with probability of not achieving glycemic control; e.g odds ratio for South Asians was 1.7 (95% CI, 1.5, 1.9), compared to individuals from high-income Europe.

Conclusion: Ethnicity is a strong determinant of glycemic control. Several non-Western populations should be targeted for interventions.



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Trends in diabetes, obesity, hypertension over twelve years in Turkey: rate of increase in diabetes is higher than the rate of increase in obesity

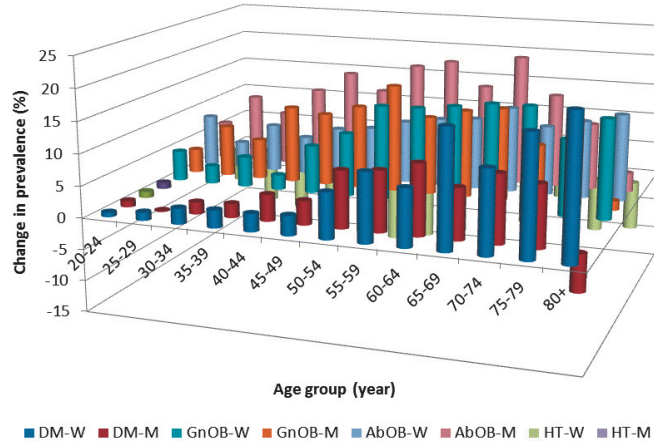
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Background and aims: According to the sixth edition of Diabetes Atlas, highest prevalence of diabetes in Europe is in Turkey. We aimed to investigate the reasons of this epidemic by comparing 12-year trends in prevalence of diabetes, IGT, obesity, abdominal obesity and hypertension in two population-based surveys in Turkey.

Materials and methods: TURDEP-I (1998) and TURDEP-II (2010) have been conducted in the same 540 centers twelve years apart in Turkey. Both surveys covered randomized people 20 years and over; TURDEP-I (n=24,714; 55.2% women) and TURDEP-II (n=25,120; 63% women). Diabetes has been defined as previously known diabetes plus new diabetes based on OGTT 2-hPG ≥200 mg/dL.

Results: Between the two surveys the mean age of the population increased by 4 years in men and women in average. The rates of increase in diabetes, IGT, obesity and abdominal obesity in men were higher in women (diabetes: 1.2 vs. 0.9 fold, IGT: 2.5 vs. 2.2 fold, obesity: 1.0 vs. 0.4 fold, abdominal obesity: 1.0 vs. 0.3 fold; p<0.001). Hypertension increased by 9% in men while decreased by 12% in women (see Figure). Absolute change in the prevalence of diabetes is correlated to the absolute change in both general (r=0.74, p<0.001) and abdominal obesity (r=0.54, p=0.004) but not to hypertension. The correlation does not change when the two populations are controlled for sex and age (general obesity: r=0.65, p=0.001, abdominal obesity: r=0.71, p<0.001). **Conclusion:** Diabetes increased in epidemic proportion over the recent 12-year period in Turkey. Although the contributions from aging of the population and obesity are remarkable, these factors could not explain the whole problem; other lifestyle factors (such as change in nutrition and physical activity habits) should be examined in detail to find the causes of diabetes epidemic in Turkey.

Figure: Absolute change in diabetes, general obesity, abdominal obesity and hypertension



Supported by: TUBITAK, SEMT, Istanbul University

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Epidemiology of hyperglycaemia in pregnancy and gestational diabetes in the World Health Organisation European region

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Background and aims: Hyperglycaemia is one of the most prevalent metabolic disorders that occur during pregnancy. The International Diabetes Federation (IDF) has developed a methodology for generating estimates of the prevalence of hyperglycaemia in pregnancy (HIP), including gestational diabetes, among women of childbearing age (20–49 years). The IDF defines HIP to include both hyperglycaemia first detected in pregnancy (HFDP) as well as previously known diabetes. The IDF uses the WHO definition of people with HFDP to include people with gestational diabetes (GDM) and also those first diagnosed with diabetes in pregnancy (DIP).

Materials and methods: PubMed and cited literature were reviewed, to identify studies reporting prevalence of HIP. A simple scoring system was developed to characterise studies. Age-specific prevalence data from studies were entered to produce estimates for five-year age groups using logistic regression to smooth curves, with age as the independent variable. Adjustments were then made to align with recently published diagnostic criteria as defined by the WHO for hyperglycaemia first detected in pregnancy.

Results: We have previously presented an analysis of the global burden of HIP and we now present a more detailed analysis of the HIP burden in the WHO-EUR-Region. After scoring and exclusion requirements, 13 studies were selected representing 11 countries (Belgium, France, Hungary, Ireland, Israel, Netherlands, Norway, Poland, Spain, Turkey and United Kingdom). The majority of studies (85%) were conducted in high income countries, only two were conducted in upper middle income countries and no studies were available for lower middle and low income countries within the Region. 54% of the selected studies were conducted in a single hospital, 31% were con-

ducted in a locally representative sample leaving only a study from Hungary as being regionally representative and a study from Turkey being nationally representative. It is estimated that in 2013 of a predicted 10.7 million live births to women aged 20–49 years 15.2% were affected by HIP. Thus, within the Region, 1.7 Million live births were affected by HIP. While the highest prevalence of HIP is found in pregnant women >40 years of age, the largest numbers of cases occur in women aged 25–35. Within the countries estimated age-standardised prevalence of HIP ranged from 5.0% of all live births affected in Belgium up to 32.1% of all live births affected in Spain.

Conclusion: Prevalence estimates of HIP are sensitive to the data from which they are derived. Within the WHO-EUR-Region there is a lack of nationally representative studies on the prevalence of HIP, especially in lower middle income countries and low income countries. More data are needed, in particular from low income countries, to strengthen the methodology. These are the first estimates of HIP in the WHO - EUR Region and conform to the new WHO recommendations regarding diagnosis. They indicate the importance of this issue from a public health and maternal and child health perspective.

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Prevalence of metabolic syndrome and cardiometabolic risk factors in patients with long-duration psychotic illnesses

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Background and aims: Patients with severe mental illness have significantly reduced life expectancy with increased cardiovascular events and a high prevalence of diabetes. As a preliminary step to testing interventions to try and improve physical health outcomes in patients with established psychosis we have conducted this study to determine the prevalence of the component risk factors that comprise the metabolic syndrome in a large cohort of established patients.

Materials and methods: Cross-sectional study of 450 patients with established (multi-episode) psychosis recruited at five centres in the United Kingdom. Anthropometric measures (height, weight and waist circumference), and blood pressure were recorded for all subjects. In addition blood samples for HbA1c, fasting glucose and lipid profile were obtained from 296 subjects.

Results: Mean age of the participants was 43.6 years (SD=10.1) and mean duration of psychotic illness was 15.7 years (SD=10.3). 57% of participants were male. 55% percent (n=238) were Caucasian and 33% (n=146) were of Black African or Black Caribbean ethnicity. 50% of participants were obese (body mass index, BMI>30) 44% were prescribed dibenzodiazepine antipsychotic medication (clozapine and olanzapine). There was no association between dibenzodiazepine use and BMI. Of the 296 participants with complete data 57% fulfilled the International Diabetes Federation (IDF) criteria for diagnosis of the metabolic syndrome. Prevalence of central obesity was very high with 82%, of participants exceeding IDF cut-off values for waist circumference. In addition, 54% had blood pressure above 130/85, 57% triglyceride above 1.7 mmol/l and 53% had HDL cholesterol below the IDF cut-off values of under 1.03 or 1.29 mmol/l for men and women respectively. 31% had fasting dysglycaemia (fasting glucose of 5.6 mmol/l or greater) including 26% fulfilling criteria for a diagnosis of diabetes.

Conclusion: These data demonstrate a high prevalence of metabolic syndrome and dysglycaemia in a relatively young population with longstanding psychosis and the presence of multiple adverse cardiometabolic risk factors that could be ameliorated to improve cardiovascular outcomes. The prevalence of central obesity is considerably greater than that of obesity diagnosed by BMI indicating a need for waist measurement to predict risk in this population.

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PS 006 Epidemiology of diabetes: comorbidities and complications

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Prevalence of undetected thyroid disorders in subjects with diabetes in South India: a cohort analysis

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Background and aims: Thyroid function test is not a part of routine diabetes practice in India, due to the extra cost involved and the lack of time for the physician to convince the patient on the requirement of an extra expensive investigation. Many a time, symptoms of hypothyroidism in diabetes patients are mistaken for hypoglycaemia and associated metabolic syndrome and hence goes grossly undetected.

Materials and methods: We screened T2DM patients, presenting at our comprehensive diabetes speciality clinic for the first time, to evaluate the prevalence of thyroid disorders. Screening was done during the past 5 years, using the 'sensitive TSH test' in Cobas E411 Elecsys. Patients with undetected hypothyroidism reported mood changes, anxiety, anger, memory loss etc. which were corrected with thyroid supplementation.

Results: During selection of 7402 consecutive T2DM subjects aged ≥ 21 yrs (62.48% male), 5.83% self reported hypothyroidism; 29.67% showed abnormal TSH values, comprising of 10.42% with TSH values suggestive of sub-clinical hypothyroidism ($3.04\text{--}10\text{ }\mu\text{IU/mL}$); 17.09% overt hypothyroidism ($>10\text{ }\mu\text{IU/mL}$) and 2.16% with low TSH values suggestive of hyperthyroidism ($<0.05\text{ }\mu\text{IU/mL}$). Abnormal TSH values showed significant association with female sex and >60 yrs age group; whereas distribution of other sub-groups were not statistically significantly different. Among females, 9.09% had mildly increased TSH levels ($3.04\text{--}10\text{ }\mu\text{IU/mL}$), 22.14% had high TSH levels ($>10\text{ }\mu\text{IU/mL}$) and 1.45% had low TSH levels ($<0.05\text{ }\mu\text{IU/mL}$). Overall, subjects with newly detected hypothyroidism showed a trend of higher BMI (1.7 kg/m^2) compared to others, but this was not statistically significant ($p>0.05$). The level of diabetes control was possibly confounded by treatment received and HbA1c did not significantly differ among hyperthyroid, euthyroid & sub-clinically hypothyroid patients (mean HbA1c=8.9%).

Conclusion: Considering the negligible extra cost of 1.26Euros for 90 tablets (presuming an average 50mcg/day dosage) of thyroid supplementation, diabetes patients will tremendously benefit from early detection of thyroid disorders. The very high prevalence of hypothyroidism in diabetes, significantly higher than in the general population, makes it imperative to include TSH test in routine diabetes evaluation in developing countries like India.

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Prediabetes is associated with early changes in microcirculation

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Background and aims: Microangiopathy in patients with type 2 diabetes (T2D) results from previous microcirculation abnormalities (e.g. increased permeability, disturbance of intracapillary pressure and blood flow). Hyperglycemia as well as hesitance of glucose level in patients with prediabetes (impaired glucose tolerance and impaired fasting glucose) have negative impact on microvessel status. The aim of the study was to investigate microcirculation in patients with prediabetes.

Materials and methods: We included 131 patients with average age 49.03 ± 8.76 years. Patients were divided into 2 groups: group 1 - 37 patients with prediabetes, group 2 - 35 patients with type 2 diabetes (with duration of disease no longer as 5 years and treated with oral blood glucose lowering drug) and group 3 - 59 almost healthy person. Microcirculation was measured by computer based conjunctival biomicroscopy (Malaja et al.), results were evaluated by the set of criteria for quantitative evaluation of conjunctival microcirculation: FC (number of active capillary tubes), AVA (arteriovenous anastomosis), Mean (vascular tortuosity), SI (sludge), Mtr (microthrombosis). Severity of each criteria was scored and more sever changes had higher degree.

Results: Microcirculation abnormalities were revealed in patients with prediabetes: we registered statistically significant decrease of active capillary tubes (FC) ($3.0[2.0;3.0]$ vs $2.0[2.0;3.0]$ in control group) ($P1\text{--}3<0.025$), increased

number of AVA (2,0 [2,0; 4,0] vs 2,0 [2,0; 2,0] in control group) ($P1-3 < 0,025$) and Mtr (1,0 [1,0; 2,0] vs 0,0 [0,0; 1,0] in control group) ($P1-3 < 0,001$). Hence in patients with prediabetes we observed hypoperfusion and microthrombosis that predispose vascular wall to atherosclerosis. We registered more significant changes in patients with T2D compared to patients with prediabetes and control group. Patients with T2D had more significant Mean (1,0 [1,0; 2,0]) compared to group 1 and 3 (1,0 [1,0; 1,0]) ($P1-2 < 0,05$ and $P2-3 < 0,001$ correspondingly), erythrocyte properties are also changed that is presented in sludge formation (2,0 [2,0; 4,0] vs 2,0 [2,0; 2,0] in groups 1,3) ($P1-2 < 0,05$, $P2-3 < 0,001$ correspondingly). Consequently microcirculation abnormalities in patients with prediabetes and T2D consist in changes as in vessel wall so and in intravessel homeostasis.

Conclusion: Analysis of microcirculation demonstrate presence of changes in microvessel system during early disturbance of glucose metabolism (that is in prediabetes). T2D is associated with more significant changes in microcirculation. Damage of microvessel is one of the factors that leads to endothelial dysfunction and atherosclerosis.

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Impact of glucose metabolism across the life course on retinal microvasculature architecture: the young Finns study

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Background and aims: Studies in healthy adults and children indicate that retinal venular abnormalities are unrelated to arteriolar changes, and may be influenced by obesity, increased inflammation and glucose metabolism. Limited research has been conducted across the life course. We hypothesized that fasting plasma glucose (FPG) in late childhood/adolescence and change in FPG from late childhood/adolescence to mid adulthood would be associated with adverse changes in retinal microvascular architecture.

Materials and methods: The Cardiovascular Risk in Young Finns Study included children aged 3 to 18 years, from five Finnish University cities, with participants chosen randomly from the national population registrar from those areas. Complete data were available for 1054 participants with a gestation ≥ 37 weeks. Retinal microvascular measures included retinal arteriolar and venular diameters.

Results: The mean age of the participants was 41 years (range 34–49 years) at the time of retinal photography. Regression analysis showed a strong positive association between change in FPG from late childhood/adolescence to mid adulthood with venular diameter (regression coefficient (β) 0.207; $p < 0.031$) and no association with late childhood/adolescence or adult FPG (β -0.092; $p = 0.391$ and 0.131; $p = 0.168$ respectively), adjusted for age and sex. No association was evident between FPG and arteriolar diameter.

Conclusion: This is the first study to assess the impact of glucose metabolism across the life course on retinal microvascular architecture. Retinal arterioles and venules are differentially associated with FPG. Change in FPG from late childhood/adolescence to mid adulthood may adversely affect the microcirculation, with important implications for cardiovascular risk.

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Serum high-sensitivity C-reactive protein levels are associated with high risk of development of diabetic nephropathy among Japanese type 2 diabetes patients

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Background and aims: Diabetic nephropathy is a common complication of diabetes and the leading cause of end-stage renal disease in developed countries. An understanding of the clinical characteristics and risk factors associated with diabetic nephropathy is useful for establishing effective therapeutic strategies to prevent the progression of diabetic nephropathy. Many cross-sectional studies revealed that the C-reactive protein (CRP) levels were high in patients with diabetic nephropathy. Only one previous study reported that baseline C-reactive protein (CRP) levels were associated with a subsequent

increment of urinary albumin excretion in patients with type 2 diabetes, but this study did not examine that either of progression or development of diabetic nephropathy was associated with high levels of CRP. The present study assessed the prospective association between baseline serum high-sensitivity CRP (hs-CRP) concentration and the subsequent risk of the development or progression of diabetic nephropathy among Japanese type 2 diabetes patients. **Materials and methods:** Longitudinal data were obtained from 2,256 patients with type 2 diabetes registered in a Japanese diabetes registry. To assess the independent associations between serum hs-CRP quartiles at baseline, and either the development or progression of diabetic nephropathy at 1-year follow-up, we used the Cox proportional hazards model adjusted for potential confounders.

Results: Mean patient ages, BMI and HbA1c levels were 66.2 years, 24.6 kg/m² and 7.5%, respectively. Firstly, we examined the association between baseline hs-CRP levels and subsequent risk of the development of diabetic nephropathy. Over the median follow-up of 0.93 years, we observed 199 cases who developed diabetic nephropathy (incidence ratio = 156.4/1000 person-years [95%CI, 137.0 to 177.6]). Baseline serum hs-CRP levels were significantly associated with the urinary albumin-creatinine ratio (UACR) at baseline ($p < 0.001$). Multivariable adjusted hazards ratios for progression from normoalbuminuria to microalbuminuria were 1.25 (95% CI, 0.74 to 2.1), 1.47 (95% CI, 1.06 to 2.05; $p = 0.022$) and 1.31 (95% CI, 1.03 to 1.66; $p = 0.025$), respectively, for the 2nd, 3rd and 4th quartile of serum hs-CRP levels, showing a statistically significant linear trend across categories ($p = 0.001$). Next, we examined the association between baseline hs-CRP levels and subsequent risk of progression of diabetic nephropathy. Over the median follow-up of 0.94 years, we observed 109 cases whose diabetic nephropathy progressed (incidence ratio = 101.0/1000 person-years [95%CI, 83.7 to 120.6], Table 2). We did not observe a significant association between hs-CRP levels and subsequent risk of diabetic nephropathy (p for trend = 0.514).

Conclusion: We observed a significant association between serum hs-CRP levels and baseline UACR levels. Serum hs-CRP levels were associated with the subsequent risk of developing, not progressing, diabetic nephropathy in type 2 diabetes patients independent of possible confounders. Serum hs-CRP may be useful for predicting patients' risk of developing diabetic nephropathy.

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Decrease in the incidence of renal replacement therapy for diabetes mellitus in the Netherlands

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Background and aims: Diabetes mellitus (DM) is considered the main cause of end stage renal disease (ESRD) in many countries. In general, its frequency is estimated to be about 40% of ESRD and would be expected to rise in parallel with the prevalence of DM. Aim of the present study was to investigate trends in incidence and prevalence of DM as cause of renal replacement therapy (RRT) for ESRD in the Netherlands in the period 2000–2012.

Materials and methods: Using the RENINE-database, a countrywide and 100% registration of subjects on RRT, the incidence and prevalence of all Dutch individuals initiating RRT having DM as primary diagnosis were obtained. The age- and gender adjusted incidence and prevalence were calculated. Trends in time were analysed with Joinpoint regression.

Results: The prevalence of DM in the Dutch general population (GP) increased from approximately 500,000 in 2000 to 893,000 in 2011. The number of individuals who started DM related RRT remained stable: 17.4 per million population (pmp) in 2000 and 19.1 pmp in 2012 with an annual percentage change (APC) of 0.8% (95% confidence interval (CI) -0.4;2.0). However, for RRT due to T1DM the incidence decreased from 7.3 pmp in 2000 to 3.5 pmp in 2012 with an APC of -4.8% (95%CI -6.5;-3.1). For T2DM it increased from 10.1 pmp in 2000 to 15.6 pmp in 2012 with an APC 3.1% (95%CI 1.3;4.8). The incidence of RRT due to unknown or missing causes of renal failure increased from 21.2 pmp to 28.5 pmp. The prevalence of RRT for DM increased at a lower rate after 2009: APC 1.0% (95%CI -0.4;2.5) versus 5.8% (95%CI 5.6;6.1). Compared to a non-DM reference population, patients on RRT due to T1DM and T2DM had an increased mortality, age and gender adjusted

hazard ratios: 1.8 (95%CI 1.7;2.0) and 1.4 (95%CI 1.3 - 1.4). Compared to the period of 2000–2004, patients initiating RRT in 2005–2009 had a lower mortality, age and gender adjusted hazard ratios: 0.8 (95%CI 0.7;0.8).

Conclusion: The incidence of RRT for DM is stable over the last decade reflecting a decrease for T1DM and an increase for T2DM. Taken together with a steady increase in prevalence of DM in the GP this may suggest that physicians may be more successful in the prevention of diabetes related ESRD

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Cardiovascular events and all-cause mortality: associations with renal function in patients with type 2 diabetes

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Background and aims: Diabetes and chronic kidney disease are independent predictors of mortality and cardiovascular (CV) events. The aims of this study were to quantify the risk of death and CV events associated with estimated glomerular filtration rate (eGFR) and to identify other potential risk factors in patients with type 2 diabetes.

Materials and methods: In this retrospective study, data were collected from The Health Improvement Network, a UK primary care database. From a cohort of patients aged 20–90 years with type 2 diabetes, 57 946 individuals were identified who had a valid serum creatinine measurement recorded in 2000–2005. Patients were followed up from their start date (date of first ever valid recorded creatinine measurement) until they met one of the following endpoints in three separate analyses: myocardial infarction (MI), ischaemic stroke or transient ischaemic attack (IS/TIA), or death; patients were censored when they reached 90 years of age or the end of the study (31 December 2010). Individuals with a record of haemodialysis or renal transplant before their start date were excluded or censored during follow-up. Incidence rates for death, MI and IS/TIA were calculated overall and by eGFR subgroup at baseline (15–29, 30–44, 45–59 and ≥ 60 mL/min). Cox regression models adjusted for potential confounding factors were used to estimate hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) for death, MI and IS/TIA according to sex, age, eGFR category and duration of diabetes.

Results: Median follow-up times were 6.80, 6.64 and 6.56 years for death, MI, and IS/TIA, respectively. Overall incidence rates for death, MI and IS/TIA were 43.65, 9.26 and 10.39 cases per 1000 person-years, respectively. Incidence rates were highest for patients with the lowest eGFRs (15–29 mL/min): 210.01, 31.65 and 32.48 cases per 1000 person-years for death, MI and IS/TIA, respectively. For patients with an eGFR ≥ 60 mL/min, the corresponding incidence rates were 31.99, 7.44 and 8.65 cases per 1000 person-years. A low eGFR (15–29 mL/min) was associated with an increased risk of death (HR: 4.00; 95% CI: 3.69–4.33), MI (HR: 3.29; 95% CI: 2.68–4.04) and IS/TIA (HR: 2.47; 95% CI: 2.01–3.03) relative to eGFR ≥ 60 mL/min (Table). The risk of death, MI and IS/TIA significantly increased with age: HRs for patients aged 75 years or older relative to patients aged 20–49 years were 12.14 (95% CI: 10.79–13.67), 3.33 (95% CI: 1.79–3.98) and 6.64 (95% CI: 5.44–8.11), respectively. Longer duration of diabetes was associated with an increased risk of death or experiencing a CV event: HRs for patients who had had diabetes for more than 15 years relative to those who had had diabetes for less than a year were 1.74 (95% CI: 1.59–1.90) for death, 1.87 (95% CI: 1.54–2.26) for MI and 1.64 (95% CI: 1.36–1.98) for IS/TIA.

Conclusion: In patients with type 2 diabetes, the risks of death, MI and IS/TIA increased with age, duration of diabetes and decreasing eGFRs.

Table Risk of death, MI and IS/TIA according to eGFR at baseline

eGFR (mL/min)	Death HR ^a (95% CI)	MI HR ^a (95% CI)	IS/TIA HR ^a (95% CI)
15–29	4.00 (3.69–4.33)	3.29 (2.68–4.04)	2.47 (2.01–3.03)
30–44	2.24 (2.14–2.35)	2.22 (1.99–2.49)	1.55 (1.38–1.73)
45–59	1.34 (1.29–1.39)	1.41 (1.30–1.54)	1.20 (1.11–1.30)
≥ 60	1 (–)	1 (–)	1 (–)

eGFR, estimated glomerular filtration rate; HR, hazard ratio; IS, ischaemic stroke; MI, myocardial infarction; TIA, transient ischaemic stroke.

^aAdjusted for sex, age and duration of diabetes.

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Factors associated with cognitive impairment in patients with newly diagnosed type 2 diabetes: a cross-sectional study

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Background and aims: Type 2 diabetes (T2DM) is a major risk factor for cognitive impairment (CI) and the pandemic of T2DM is expected to increase the global dementia burden. The factors associated with CI in T2DM are poorly understood. A limitation of previous cohorts examining CI in T2DM is the variable and long duration of T2DM among participants, which increases the risk of effects being obscured by differential survival and diabetes complications. By analysing a population with newly diagnosed T2DM, we aimed to identify modifiable factors for cognitive decline at an earlier stage in the natural history of T2DM. Our primary hypothesis was that markers of glucose dysregulation would be associated with cognitive impairment and secondary hypotheses were that i) depression, ii) black or South Asian ethnicity and iii) body mass index (BMI) would be independently associated with CI in T2DM.

Materials and methods: We performed a cross-sectional analysis of baseline measures from the South London Diabetes (SOUL-D) cohort, a population-based multi-ethnic study of individuals diagnosed with T2DM in the last 6 months. We assessed CI using the 13-item Telephone Interview for Cognitive Status (TICS-M), which has a range of scores from 0–39. We defined CI as the lowest 10% of scores and the remainder as controls. We performed univariate and multivariate analyses of the association between CI and 1) markers of glucose dysregulation (HbA1c, fasting glucose, micro/macrovacular complications); 2) sociodemographic factors (age, gender, ethnicity); 3) vascular risk factors (BMI, hypertension, hyperlipidaemia, smoking) and 4) depression (PHQ-9 score >12). We used Student's t-test for continuous data and χ^2 tests for categorical data and logistic regression to control for key confounding variables, including premorbid intelligence estimated by National Adult Reading Test (NART) score.

Results: In the preliminary analyses, of 1791 patients recruited, 1680 (93.8%) had a complete TICS-M assessment. Average diabetes duration was 4.6 (SD 2.2) months. Cognitive impairment was defined as TICS-M score <17 . In univariate analyses, there were no differences in HbA1c, ($p=0.22$), fasting glucose ($p=0.96$), microvascular complications ($p=0.31$), macrovascular complications ($p=0.41$), depression ($p=0.52$), BMI ($p=0.2$), smoking ($p=0.13$), hyperlipidaemia ($p=0.21$) and hypertension ($p=0.70$) between patients with CI and controls. Patients with CI were older ($p<0.001$), more likely to be female ($p<0.001$), of black or South Asian/other ethnicity ($p<0.05$) and had lower scores on NART ($p<0.0001$). In multivariate analyses including age, gender, ethnicity, HbA1c, BMI and NART as covariates, the effects of age, ethnicity and NART remained significant but the effect of gender ($p=0.12$) was attenuated.

Conclusion: Although poor glycaemic control and depression are known risk factors for CI in established T2DM, this was not seen at diagnosis. Instead, older age, black or South Asian ethnicity and poorer NART scores were associated with CI at diagnosis of T2DM, though the latter two associations may partly reflect poorer literacy levels. Overall cognitive scores were lower than studies in non-diabetic populations. Our results suggest that at the time of T2DM diagnosis there may be a window of opportunity to identify patients at higher risk of CI.

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Risk factors for atrial fibrillation in type 2 diabetes. Report from the Swedish National Diabetes Register: NDR

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Background and aims: Atrial fibrillation (AF) is more frequent in patients with type 2 diabetes than in the general population. Further, AF has been shown to have a strong impact on risk for cardiovascular complications and mortality in patients with type 2 diabetes, who generally have elevated risks of cardiovascular complications, i.e. at least twice as common as in those with-

out diabetes. The aim was to investigate which factors that contributes to the risk of AF in type 2 diabetes using national register data from Sweden.

Materials and methods: Observational cohort study of 64,864 patients with type 2 diabetes, aged 30–79 years, without AF at baseline. A history of cardiovascular disease (CVD) was present in 16.9% and 3.6% had a history of congestive heart failure (CHF). Patients were followed-up for development of AF ($n=2,719$) during 4.2 years from 2003 to 2009. A subgroup of 52,939 patients without history of CVD or CHF at baseline was also analysed.

Results: At multivariate logistic regression, odds ratios (OR) of AF were 4.49 for a baseline history of CHF and 7.98 for in-study developed CHF. Similarly for a history of CVD, the OR was 1.28 and for in-study developed myocardial infarction an OR of 1.37. Cardiovascular risk factors associated with risk for AF were hypertension (OR, 1.42), cumulative microalbuminuria (OR, 1.24), obesity (OR, 1.31) and ORs for each decade of increasing age was 1.93, and 1.43 for male gender, with all predictors $p<0.001$. Among patients without history of CVD or CHF at baseline, significant predictors were similarly for in-study developed CHF (OR, 10.2), hypertension (OR, 1.52), cumulative albuminuria (OR, 1.25), obesity (OR, 1.34) and ORs for each decade of increasing age was 1.93, and 1.48 for male gender, with all predictors $p<0.002$. In multivariable models, the following factors were not significantly ($p>0.05$) related to AF as the outcome: HbA1c, blood lipids and smoking status. Population Attributable Risk Percent (PARp: percent cases reduced if predictors could be eliminated) for AF was with hypertension 27%, in-study CHF 25% and previous history of CHF 8%, cumulative microalbuminuria 7% and for obesity 11%.

Conclusion: Except for CHF, hypertension, albuminuria and obesity account for large portions of PAR for AF in type 2 diabetes. The study underlies the contribution of CHF, hypertension and microalbuminuria to AF development, factors that are shown to be modified by pharmacological treatment including ACE-inhibition. Further, obesity also contributes to AF and life style measures aiming at weight reduction if obese may have benefits for reduction of AF incidence. Intervention studies on AF-prevention are warranted.

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Risk of acute myocardial infarction in 121289 patients with type 2 diabetes: emphasis on socioeconomic status and ethnicity

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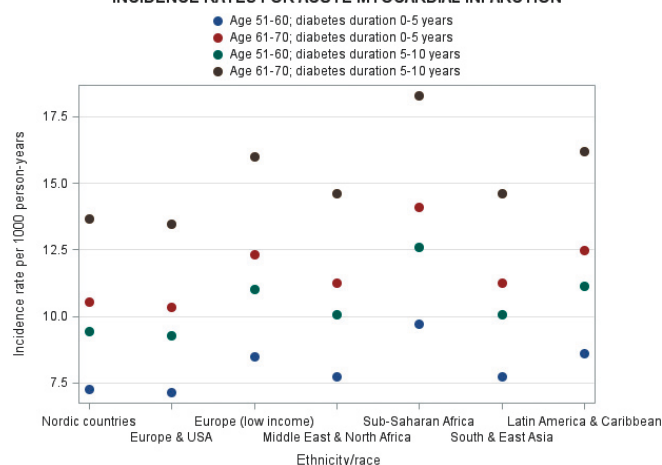
Background and aims: Little is known about ethnicity and the risk of coronary heart disease (CHD) and acute myocardial infarction (AMI) in diabetes. We examined the relationship between ethnicity and CHD death or hospitalization for AMI in patients with type 2 diabetes.

Materials and methods: Patients included in the Swedish National Diabetes Register (2002–2006) were followed until a diagnosis of AMI or CHD death, death (other causes) or December 31 2011, through the nationwide Inpatient Registry and National Death Registry. All individuals (15.5% foreign-born) had equal access to low-cost health care. The study population included individuals from all the world's continents. Detailed data on income and education was retrieved from the Swedish National Board of Health and Welfare. Incidence rates for AMI/CHD death were estimated by Poisson regression. Relative risk was estimated by Cox regressions adjusted for ethnicity, income, education, lipids, sex, age, blood pressure, smoking, kidney function, body mass index, physical activity, chronic obstructive pulmonary disease and cardioprotective drugs. Model fitting proceeded conventionally and, where appropriate, covariates were time dependent.

Results: In 121289 patients with type 2 diabetes, 12861 (10.6 %) had an event (mean follow-up 6.1 years). Incidence rates (figure) varied strikingly by ethnicity. Non-Western groups, particularly Sub-Saharan Africa and Latin America displayed particularly high figures. Adjusted hazard ratios for AMI/CHD death by ethnicity was: High-income Europe, North America, 1.10 (0.97–1.25); Low-income Europe, 1.19 (1.03–1.37); Latin America & Caribbean, 1.30 (0.90–1.88); Middle East & North Africa, 1.37 (1.16–1.62); Sub-Saharan Africa, 1.15 (0.95–1.40) and South & East Asia 1.10 (0.95–1.34), compared with Nordic countries. While education had no effect on the hazard, income was strongly associated with the risk of AMI/CHD death. Belonging to the lowest income quintile was associated with a hazard ratio of 2.25 (2.15–2.39), compared to the highest quintile. Among traditional risk factors, the hazard ratios for chronic obstructive pulmonary disease and low kidney function were surprisingly high.

Conclusion: Ethnic disparities in risk of AMI/CHD death could be explained by modifiable risk factors.

INCIDENCE RATES FOR ACUTE MYOCARDIAL INFARCTION



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Neighbourhood deprivation and inequities in mortality among patients with diabetes mellitus: a multilevel study

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Background and aims: Individuals with diabetes mellitus (DM) have higher risk of premature mortality and different mortality patterns, compared to individuals without DM. In this large-scale study, our aim was to examine if mortality (all cause and the five most common causes of death among individuals with DM in Sweden) vary by neighborhood-level deprivation after taking sociodemographic factors and comorbidities into account.

Materials and methods: The Swedish nationwide prescription register was used to identify 367 477 patients with DM who were followed for the outcome variable mortality in order to test whether neighborhood-level deprivation was significantly associated with mortality after adjusting for the following covariates: age, gender, marital status, family income, educational attainment, country of origin, mobility, urban/rural status and comorbidities.

Results: Multilevel logistic regression models were used in the statistical analyses. The odds ratio (OR) for mortality increased with increasing deprivation. The OR in high deprivation neighborhoods was 1.13 (95% CI 1.08–1.18). The five most common causes of death in the study population were coronary heart disease (CHD), stroke, psychiatric disorders, cancer and type 2 DM. For CHD and psychiatric disorders the ORs increased by increasing neighborhood deprivation and reached 1.37 (95% CI 1.26–1.48) and 1.29 (95% CI 1.04–1.61), respectively, in high deprivation neighborhoods. The corresponding OR for mortality from type 2 DM was 1.24 (95% CI 1.11–1.40).

Conclusion: This study shows that neighborhood-level deprivation is an important factor for mortality among patients with DM. Future research should examine possible pathways between neighborhood deprivation and mortality in order to reduce inequities in mortality among patients with DM.

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PS 007 Pharmaco-epidemiology

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Temporal trends in use of evidence-based treatments and risk factor control: a decade of nationwide monitoring

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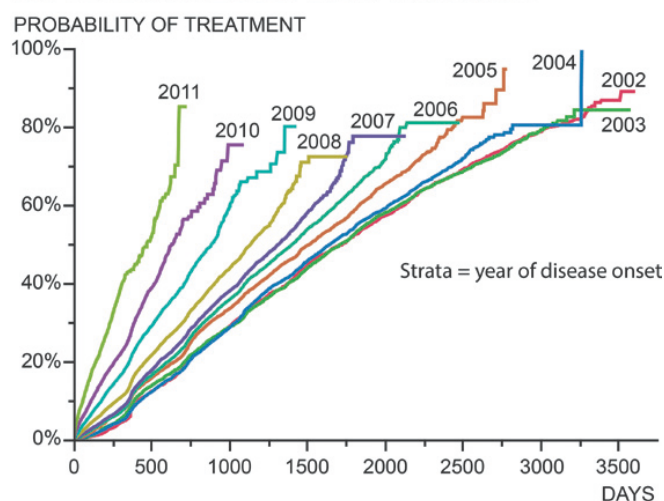
Background and aims: Glucose-lowering therapy with metformin is a cornerstone of diabetes treatment. Guidelines recommend instituting metformin at the time type 2 diabetes is diagnosed. Likewise, the primary preventive effects of statins in individuals with type 2 diabetes has been recognized for almost a decade. Little is known about the pace of adoption of these evidence-based medications. We examined this in a nationwide prospective study spanning over a decade.

Materials and methods: We used the Swedish National Diabetes Register to include 131935 newly diagnosed (within one year) cases of type 2 diabetes during 2002 to 2011. The study population was divided in ten cohorts corresponding to the year of diagnosis. To study trends in use of oral glucose-lowering drugs we included individuals who were treated with diet and lifestyle modifications at baseline. Patients were followed until initiation of oral glucose-lowering therapy or appropriate censoring. HbA1c was followed annually for each cohort. Trends in statin therapy was studied in therapy naive patients who did not experience any cardiovascular event throughout the study. Lipid markers were followed annually for each cohort. Time to event (instituting therapy) was analysed by means of Kaplan-Meier method.

Results: We included 58407 patients treated with diet and lifestyle modifications. We observed a striking pattern (figure I) towards earlier initiation of glucose-lowering drugs since 2002. Median time to drug in 2002 was 1730 (95% CI 1636, 1801) days compared to 471 (95% CI 420, 518) days in 2011. However, HbA1c levels displayed a different trend; HbA1c (at the same duration of diabetes) declined steadily from 2002 to 2007, whereafter levels increased. Considering statin therapy, we note the same pattern as with glucose-lowering drugs. Median time to therapy decreased from 2450 (95% CI 2360, 2530) days in 2002 to 650 (95% CI 580, 730) days in 2011. Interestingly, the curve for 2011 leveled off and crossed the curve for 2010. LDL-cholesterol displayed a pattern similar to HbA1c - an initial decline from 2002 to 2007 and thereafter a steady increase. Post hoc analysis of trends in body mass index (BMI) and physical activity sought to find an explanation for these trends; we noted that BMI at baseline and each point thereafter has increased annually since 2002.

Conclusion: We document a striking improvement in time to instituting oral glucose-lowering drugs. We also note that use of statins in primary prevention has accelerated in parallel. However, neither HbA1c nor lipid levels reflected these trends.

TIME TO ORAL GLUCOSE-LOWERING DRUG



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Prescribing patterns of antidiabetic drugs within the first year following diagnosis of type 2 diabetes: results from the DD2 study

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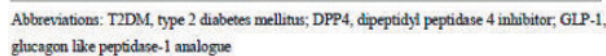
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Background and aims: Real-world data are sparse on prescribing patterns of antidiabetic drugs in cases of newly diagnosed type 2 diabetes mellitus (T2DM) and on patient characteristics that may predict type of early pharmacotherapy.

Materials and methods: We studied 1,317 newly diagnosed T2DM patients enrolled in the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort from outpatient hospital clinics and general practice, with at least one year of follow-up. We described the number and type of antidiabetic drugs prescribed in the first year after T2DM diagnosis. Using Poisson regression, we calculated risk ratios (RRs) of treatment with different antidiabetic drugs associated with baseline patient characteristics.

Results: Of the 1,317 newly diagnosed T2DM patients, 198 (15%) did not use any antidiabetic drugs within the first year post-diagnosis, 867 (66%) used only one drug, 197 (15%) used two drugs, and 55 (4%) used three or more drugs. Figure 1 demonstrates the ranking of individual types of antidiabetic drugs used by our 1,317 T2DM patients. The likelihood of receiving combination therapy with two or more drugs in the first year post-diagnosis was substantially higher in T2DM patients aged ≥ 3 were at higher likelihood of receiving combination therapy (30%; RR = 1.54, 95% CI: 1.05-2.26) than T2DM patients with no comorbidity (19%). Weight gain > 30 kg since 20 years of age and lack of regular physical exercise also increased the likelihood of receiving combination therapy during the first year post-diagnosis (RR = 1.42, 95% CI: 1.14-1.78 and RR = 1.42, 95% CI: 1.11-1.82, respectively). Higher likelihood of receiving combination therapy also was observed in T2DM patients with fasting blood glucose > 7 mmol/L at diagnosis (RR = 3.37, 95% CI: 2.43-4.68), HbA1c ≥ 7.5 (RR = 3.85, 95% CI: 3.00-4.94), and C-peptide < 300 pmol/L (RR = 1.78, 95% CI: 1.15-2.76).

Figure 1. Antidiabetic drug use in the first year following a new T2DM diagnosis among 1,317 patients enrolled in the DD2 project



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Materials and methods: Patients with a physician prescription for metformin, sulfonylurea (SU), thiazolidinedione (TZD) or dipeptidyl peptidase-4 inhibitor (DPP-4i) as monotherapy between January 1, 2009 to June 30, 2012 (index period) were selected from the IMS Disease Analyser-Mediplus UK database. Patients were required to have a diagnosis of T2DM, be ≥ 18 years old at the time of diagnosis, and have medical records available in the database for 1 year before and after the index date. Patients were excluded if they had at least one diagnosis of type 1 diabetes mellitus, their dosing regimen (once daily or twice daily) could not be determined at the index date, they switched between once- and twice-daily dosing regimens during the 12-month post-index period, or they violated the monotherapy requirement during the 12-month post-index period. Adherence was assessed using the proportion of days covered (PDC) defined as total days medications supplied divided by days in follow-up period. Logistic regression was used to assess factors (age, gender, previous treatment status [new to or previously on OAM], dosing regimen [once- or twice-daily], and number of concomitant medications) associated with whether or not a patient is adherent (yes: PDC $\geq 80\%$, no: PDC $< 80\%$).

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Conclusion: Medication adherence was shown to be suboptimal in this cohort of patients with T2DM in the UK. Younger age and use of fewer concomitant medications were factors associated with poor medication adherence in this study.

Parameter [†]	OR [‡]	95% CI
Male	0.98	0.88, 1.09
Age <45 years	0.54	0.44, 0.67
Age 45 and <65 years	0.81	0.72, 0.90
New to oral antihyperglycemic monotherapy treatment	1.07	0.94, 1.22
Twice-daily dose	1.02	0.89, 1.17
No other concomitant prescriptions	0.70	0.57, 0.87
1-2 Concomitant prescriptions	0.86	0.76, 0.97

[†]Adjusted for baseline comorbid conditions; [‡]Reference categories: female, age ≥ 65 years, previously on oral antihyperglycemic monotherapy treatment, once-daily dose, and ≥3 concomitant Rx's; *An OR >1 indicates a positive association with adherence and an OR <1 indicates a negative association with adherence.

Less adherent vs reference group More adherent vs reference group

Clinical characteristics, comorbidities, and treatment patterns among patients with new-onset type 2 diabetes in a large integrated health system

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Background and aims: The incidence of type 2 diabetes (T2D) has steadily increased over the past few decades. In the United States approximately 1.9 million people ages 20 years or older were newly diagnosed with diabetes in 2010 alone. Despite this increase, there are limited real-world data describing the clinical characteristics, comorbidities, and treatment patterns of patients with new-onset T2D. The objective of this research was to assess the profiles and treatment patterns of patients with new-onset T2D in a large integrated health system.

Materials and methods: An enterprise-wide electronic health record system (EHR) at a large U.S. academic health center was used to conduct a cross-sectional analysis of 26909 patients with new-onset T2D diagnosed between 2008 and 2012. Patients with T2D were identified using a validated algorithm to classify T2D subjects based on a combination of clinical diagnoses, medications, and laboratory results. Patients with new-onset diabetes were isolated by requiring at least 2 office encounters at the institution with a primary care provider and/or endocrinologist prior to the diagnosis of T2D. The population was divided into five strata based on the year in which the new-onset diagnosis of T2D occurred: 2008, 2009, 2010, 2011, and 2012. Comorbidities, glycemic control, and active prescriptions were assessed at 1 year after diagnosis.

Results: The population was 51.1% female and 73.5% White. A total of 4150 (15.4%), 4500 (16.7%), 5372 (20.0%), 6301 (23.4%) and 6586 (24.5%) patients were included in each of the incidence year stratum, respectively. The mean ages (years) for patients at the time of diagnosis across the incidence year strata were 62.3, 61.2, 60.8, 61.0, and 60.8, respectively. The prevalence of hypertension (HTN) within 1 year of new-onset T2D was 80%. The percentages of patients with active prescriptions for 2 or more classes of anti-diabetic agents at 1 year after diagnosis were 32.3, 32.6, 28.4, 29.5, and 31%, respectively. The selection of anti-diabetic therapy was rather consistent among the new-onset T2D patients from 2008–2012, with the only exception being a steady decline in the use of thiazolidinediones mirrored by a concordant rise

in the use of dipetidyl peptidase-4 (DPP-4) inhibitors. Interestingly, within each incidence year strata, 52.1, 47.0, 48.3, 54.2, and 55.7% of patients did not have an HbA_{1c} measurement recorded in the lab section of the EHR within the 1st year of diagnosis. Among patients with available HbA_{1c} values, the percentages of patients with inadequate glycemic control (HbA_{1c}>8%) at 1 year were 25.1%, 24.4%, 22.2%, 25.9%, and 28%, respectively.

Conclusion: A high prevalence of HTN was observed among this population of patients with new-onset T2D. Nearly 1/3 of the new-onset T2D patients were receiving 2 or more classes of anti-diabetic agents at 1 year after diagnosis. The use of thiazolidinediones in patients with new-onset T2D has decreased, whereas the use of DPP-4 inhibitors has increased. Of note, the use of sulfonylureas has not decreased, despite the availability of several new agents. Approximately 1/2 of new-onset T2D patients did not have an HbA_{1c} value recorded in the lab section of the EHR within the 1st year of diagnosis.

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Burden of baseline comorbidities in patients with type 2 diabetes initiating various classes of antidiabetic agents - an analysis of a large US claims database

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Background and aims: Channeling bias is a concern when designing observational comparative studies including users of different drug classes for the management of Type 2 Diabetes Mellitus (T2DM). Characterizing the differences in patient baseline characteristics across comparator drug classes will aid in the evaluation of channeling bias. This study described patient demographic and clinical characteristics among US patients with T2DM initiating a new class of antidiabetic drug in 2012.

Materials and methods: US adult patients with T2DM who initiated a new class of antidiabetic drug during the first six months of 2012 (N=183,692) were identified in the Truven MarketScan Commercial Claims and Encounters database. The date of first new class of oral or injectable antidiabetic drug during the study period was defined as the index date. Patient's baseline characteristics were identified in the 12 months prior to the index date. Comorbidities were based on ICD-9-CM diagnostic codes and NDC codes used for prescriptions.

Results: We identified 183,692 patients with majority (40%) initiating biguanides. Similar mean age was seen in all of the drug classes except GLP-1 RA users who were younger. Contrary to other classes, GLP-1 RA users were predominantly female. Over 50% of new users had hyperlipidemia and hypertension at baseline with the prevalence being highest in DPP-4i and GLP-1 RA users. A diagnosis of obesity was most notable in GLP-1 RA users. History of heart failure was highest in insulin users. Although rare, history of acute and chronic pancreatitis was lowest in GLP-1 RA and DPP-4i users. Compared to biguanide users, more concomitant use of lipid lowering medications was observed in users of all other classes.

Conclusion: Differences were observed in the burden of comorbidities across different antidiabetic drug classes initiated which suggests a degree of channeling bias. Careful consideration and accounting of these factors dur-

ing analysis is needed when conducting comparative observational studies among patients treated for Type 2 diabetes.

	Biguanide	SU	TZD	DPP-4i	GLP-1 RA	Insulin
	N=74264 40%	N=34617 19%	N=4712 3%	N=34057 19%	N=10119 5%	N=25923 14%
Age (Mean±SD)	57.3±12.1	59.6±12.6	58.6±11.7	59.3±11.9	55.7±10.1	59.6±13.2
Patient characteristics (%)						
Female	47.4	44.2	40.1	43.5	54.5	46.6
Retinopathy	3.0	4.5	5.2	5.3	6.1	6.8
Neuropathy	6.3	7.0	6.7	6.7	8.3	9.8
Renal disease	4.1	9.4	7.9	8.8	7.6	16.4
Hyperlipidemia	52.1	53.7	56.8	57.1	59.3	52.5
Congestive heart failure	3.2	5.6	3.2	4.8	3.2	10
Ischemic Heart Disease (MI, angina)	12.5	15.9	13.2	16.1	13.9	21.4
Hypertension	59.8	62.9	61.4	65.0	63.4	65.1
Obesity	11.3	9.6	8.8	9.6	16.5	12.0
Acute Pancreatitis	0.5	0.6	0.7	0.5	0.3	1.4
Chronic Pancreatitis	0.2	0.2	0.2	0.1	0.1	0.6
Patients on lipid lowering RXs	48.1	59.4	61.0	66.4	70.7	61.6

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Hospitalisation frequency for hypoglycaemia and emergency calls in type 2 diabetes mellitus patients exposed to vildagliptin vs insulin-secretagogues in the French Health Insurance database

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Background and aims: To compare the frequency of severe hypoglycaemic episodes leading to hospitalization and of emergency calls for any cause in patients exposed to the DPP-4 inhibitor vildagliptin vs those exposed to insulin secretagogues (IS; sulphonylurea or glinide).

Materials and methods: Retrospective data were extracted from the EGB (Echantillon Généraliste des Bénéficiaires) database: a data resource comprising a sample of ~1% of all patients registered in the French National Health Insurance system (~600,000 patients). Type 2 diabetes mellitus patients exposed to regimens comprising either vildagliptin (excluding treatment with IS, insulin or another incretin therapy) or IS (excluding treatment with insulin and any incretin therapy) between 2009 and 2012 were selected. Hospitalizations related to hypoglycaemia during the exposure periods were identified in both cohorts. Two comparative analyses adjusting for key covariates within the model (subjects matched for age, gender, socioeconomic status, drug exposure duration), or with multivariate logistic regression, were performed.

Results: 1,440 patients were exposed to vildagliptin and 10,019 to IS. Patients in the IS cohort were older than in the vildagliptin cohort (mean 67.3 (SD: 12.8) years old vs. 63.5 (SD: 11.9), p<0.0001) and had a longer exposure duration over the study period (vildagliptin has been marketed since September 2009 in France). During the observation period, no patient from the vildagliptin cohort (0.0%) was hospitalized for hypoglycaemia vs 130 (1.30%) with IS (138 hospitalizations) (p<0.0005). The rough hospitalization rates for hypoglycaemia were 0 in the vildagliptin cohort vs 5.6 hospitalization/1000 patient-years [95% CI: 4.7; 6.6] in the IS cohort (p=0.02). 60 patients in the vildagliptin cohort (4.2%) vs 2,144 patients (21.4%) in the IS cohort visited the emergency department (p<0.0001), but with no significant difference between the 2 cohorts regarding the rates of all emergency calls/1000 patient-years (p=0.5). After adjustment, rates of hospitalization and of all emergency calls were significantly lower with vildagliptin vs IS (Table). Consistent results were found when considering treatment initiations only, in the IS cohort. Due to limitations in the availability of some data in the database, adjustments did not consider HbA_{1c} levels or other potential confounding factors.

Conclusion: There was a significantly lower frequency of hospitalization for severe hypoglycaemia and of all emergency calls in patients exposed to vildagliptin vs IS. These real-life data should be taken into consideration in the benefit/risk evaluation of the drugs.

Table : Adjusted comparison of the frequency of events in the 2 cohorts

	Vildagliptin (N=1,440)	SU/Glinide (N=10,019)	p-value
Patient-years (PY) exposure	581	4,403	
No. of patients with hospitalization for hypoglycaemia	0 (0.0%)	58 (0.59%)	p=0.0034
No. of hospitalizations for hypoglycaemia	0	60	
Adjusted rates of hospitalizations for hypoglycaemia/1000 PY	0.0 [0.0; 0.0]	13.6 [10.4; 17.5]	p=0.0003
No. of patients with emergency calls (any cause)	60 (4.20%)	823 (8.21%)	p<0.0001
No. of emergency calls	77	1,035	
Adjusted rates of emergency calls/1000 PY	132.6 [104.6; 165.6]	235.0 [220.9; 249.8]	p<0.0001

Supported by: Novartis SAS

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Predicting glycaemic changes in the non-diabetic general population: a DIRECT study

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Background and aims: Recently, glycated haemoglobin (HbA1c) levels have been introduced as a diagnostic criterion for type 2 diabetes. Our study evaluates whether in addition to baseline HbA1c levels, non-invasive measures can be used to predict changes in HbA1c levels during 6 years follow-up.

Materials and methods: Data from 2,887 initially non-diabetic subjects (based on ADA 2011 criteria) from 3 population-based cohorts (Hoorn Study, Inter99, KORA S4) were used to develop sex-specific linear regression models predicting change in HbA1c levels during follow-up. By using change in HbA1c levels, lab differences were avoided. To minimize overfitting of the model, we performed internal validation using bootstrapping techniques. Calibration was assessed with calibration graphs. Discriminative performance was assessed with classification tables, dichotomizing HbA1c levels ($\geq 5.7\%$ vs $< 5.7\%$).

Results: At baseline, mean HbA1c level was 5.6%. During a mean follow-up of 6 years (SD: 0.7 years), median change in HbA1c levels was +0.02%. HbA1c levels increased in 51% of the subjects, 2.3% of the subjects developed HbA1c levels $\geq 6.5\%$. After backward selection, next to baseline HbA1c levels, for men: age, waist circumference, smoking, and parental history of diabetes were retained in the prediction model (explained variance (R²): 33%); and for women: BMI and waist circumference (R²: 23%). Calibration plots showed good agreement between predicted and observed HbA1c levels at follow-up. With respect to discrimination, our model classified 75% of the subjects correctly as having high/low HbA1c levels.

Conclusion: In the non-diabetic population, non-invasive predictors can be used next to baseline HbA1c levels to predict change in HbA1c levels during 6 years follow-up. This model can be used in clinical practice to determine which patients are at high risk of glycaemic deterioration. High-risk patients can then be monitored more regularly than low-risk patients and targeted preventive interventions can be initiated.

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Factors associated with weight gain and hypoglycaemia and the impact upon hospitalisation in type 2 diabetes patients managed with metformin plus sulfonylurea

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Background and aims: The importance of avoiding complications that decrease quality of life and consume healthcare resources in people with type 2 diabetes is well understood. However, the relationship between resource utilisation and patient phenotype is less well researched. The objective of this study was to assess factors associated with weight gain and the occurrence of hypoglycaemia in type 2 diabetes (T2DM) patients managed with metformin plus sulfonylurea (M+S), and any associated impact upon hospital resource utilisation.

Materials and methods: The study was a retrospective cohort study using the UK Clinical Practice Research Datalink (CPRD) and the Hospital Episode Statistics (HES) database. The cohort analysed were those with T2DM treated with M+S for at least 3 months during the period 1/1/2001 to 31/12/2011; patients with a diagnosis of malignant disease were excluded. The association between phenotypic factors at baseline (therapy escalation from metformin to M+S) and weight gain (defined as $> 2\text{kg}$ weight change over 12 months) and primary care recorded hypoglycaemia (≥ 1 episode) over 12 months following therapy escalation was assessed using logistic regression. Hospitalisation associated with increasing body mass index (BMI) and hypoglycaemia was also assessed. Analysis was undertaken using R version 2.12.2

Results: A total of 11,071 patients met the study inclusion/exclusion criteria with mean age at baseline of 60.7 (SD=11.4) years, 39% female, 5.4 (SD=4.0) years duration of diabetes, HbA1c 8.7% (SD=1.6), weight 92.2 kg (SD=19.6) and BMI 32.2 kg/m² (SD=6.1). Weight gain (1=weight gain; 0 otherwise) was observed in 28.35% (n=3,139) and was significantly associated with baseline age (OR=0.99 for one-year increase in age, $p<0.01$), female gender (OR=0.87, $p<0.05$) and baseline weight (OR=1.003 for 1 kg increase in weight, $p<0.05$) and HbA1c (OR=1.06 for 1% increase in HbA1c, $p=1$ episode; 0 otherwise) occurred in 1.3% (n=142) of patients and was associated with duration of diabetes (OR=1.04 for one-year increase in duration, $p<0.05$), baseline HbA1c (OR=0.86 for 1% increase in HbA1c, $p<0.05$) and prior complications status (OR=1.92, $p<0.05$). Hospitalisation occurred in 10% of patients (n=1,125) and was significantly associated with BMI (OR=1.02 for 1 kg/m² increase in BMI, $p<0.01$) but not hypoglycaemia. The mean number of hospital admissions over the follow-up period was 1.7, 1.8, 1.9 and 3.1 in those with BMIs at the time of admission in the normal, overweight, obese and morbidly obese categories respectively.

Conclusion: This real-world observational analysis suggests there are identifiable phenotypic characteristics predictive of weight gain, and there are identifiable phenotypic characteristics predictive of hypoglycaemia. This study also shows a general relationship between increasing BMI and hospitalisation, most notably in the comparison of normal vs morbidly obese subjects, that may not be adequately captured in widely used vascular risk equations such as UKPDS in which BMI has minimal influence on risk. Consequently the value of diabetes management strategies that minimise weight gain may be underestimated.

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Analysis on incidence of new-onset diabetes mellitus after renal transplantation in different eras

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Background and aims: To retrospectively evaluate the long-term fluctuation of fasting plasma glucose (FPG) after renal transplantation, in order to explore the incidence of new-onset diabetes mellitus after transplantation (NODAT) in different eras.

Materials and methods: We retrospectively evaluated 709 patients receiving kidney transplantation at our center between 1 January, 1993 and 31 December, 2008. After excluding patients with uncompleted data, graft failure or death within 1 year after transplantation, multi-organ transplant recipients, transplant more than once or previously known diabetes, 428 patients were analyzed. The incidence of new-onset diabetes mellitus after renal transplantation was analyzed according to FPG in different eras. Immunosuppressive treatment after transplantation might be cyclosporine-A,(CSA)+ mycophenolate mofetil(MMF)(or Azathioprine (AZA)) + glucocorticoid or tacrolimus (FK506)+MMF(or AZA)+ glucocorticoid. We use AZA for anti-proliferative drug before 1997 and MMF after that. We started using FK506 in 1999 and in some patients using CD25 monoclonal antibody for immunosuppression induction since 2001. Patients were divided into three groups 1993-1996, 1997-2000, 2001-2008 according to different immunosuppressive treatment, and the incidence of NODAT was compared between different eras.

Results: Of the 428 Patients, 87 developed NODAT (20.3%) during a mean follow-up of 6 years. According to the different immunosuppressive treatment in the different eras, patients were divided into three groups 1993-1996, 1997-2000, 2001-2008, of which the incidence of NODAT was 18.42%, 17.46% and 21.11% respectively. There was no difference in the incidence of NODAT among three transplantation eras.

Conclusion: The incidence of NODAT was 20.3% in patients surviving for more than 1 year during a mean follow-up of 6 years at Zhongshan Hospital. There was no difference in the incidence of NODAT among three transplantation eras.

PS 008 Type 1 diabetes: genes and biomarkers

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Primary autoantigen specific genetic traits in the pathogenesis of type 1 diabetes

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Background and aims: Natural history studies on type 1 diabetes (T1D) associated autoimmunity provide an opportunity to identify detailed mechanisms of autoantigen specific genetic traits of diseases pathogenesis. We hypothesized that if the effect of a specific T1D-risk associated genetic marker is related to the triggering autoantigen of beta-cell destruction, the effect of the single variant should be dependent on the first appearing autoantigen-specific autoantibody.

Materials and methods: The study subjects were participants in the prospective DIPP study and carried T1D-risk associated HLA class II genotypes. From the DIPP study cohort, we were able to identify 170 subjects with IAA as the first biochemical autoantibody and selected 325 autoantibody negative controls matched for gender, date of birth and study center. Similarly we identified 151 subjects with GADA as the first biochemical autoantibody and 285 autoantibody negative controls for them. Forty-three single nucleotide polymorphisms (SNPs) associated with T1D risk were genotyped using the Sequenom platform. Differences in effect of various SNPs on the development of autoantibodies and further progression to clinical T1D were tested among subjects with IAA or GADA as the first biochemically-defined autoantibody. Cox regression analysis was performed to test the effect of the gene markers on the T1D pathogenesis.

Results: The effect of INS rs689, IKZF4 rs1701704 and ERBB3 rs2292239 polymorphisms differed significantly between subjects with IAA or GADA as the first biochemical autoantibody. INS SNP strongly affected the development of clinical T1D and, in particular, the appearance of beta-cell humoral autoimmunity among the group with IAA as the first autoantibody ($p=0.0054$ and 0.0024 , respectively) whereas no effect of the INS SNP on the T1D pathogenesis in the GADA group could be observed. In contrast, IKZF4 and ERBB3 SNPs were associated with the development of T1D in the GADA group ($p=0.0022$ and 0.0064 , respectively) and, the two polymorphisms affected the progression rate of beta-cell destruction after the appearance of autoimmunity in the GADA group ($p=0.0025$ and 0.0024 , respectively) whereas no effect of these SNPs on the appearance of T1D or the progression rate of beta-cell destruction could be observed in the IAA group.

Conclusion: The first appearing autoantibody specific approach revealed two genetic pathways dependent on the primary autoantigen. The effect of INS gene SNP on the pathogenesis of T1D was restricted to subjects with IAA as the first autoantibody whereas the SNPs in the IKZF4-ERBB3 locus increased the T1D risk and, more specifically, the progression rate of beta-cell destruction among subjects with GADA as the first autoantibody.

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Concentrations of soluble receptor for AGEs decline at seroconversion in children with preclinical type 1 diabetes but not in autoantibody positive non-progressors

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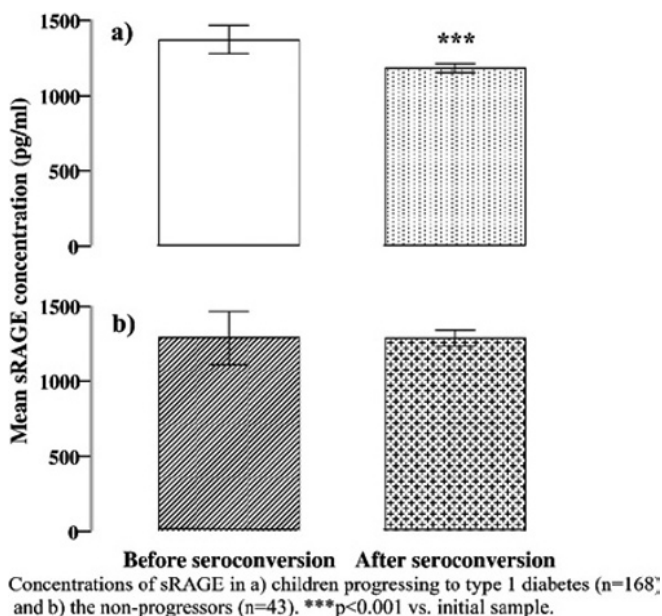
Background and aims: Our previous studies suggest that concentrations of soluble receptor for advanced glycation end products (sRAGE) might play a

role in the process leading to type 1 diabetes. We set out to define the changes in sRAGE in prediabetic and autoantibody positive children.

Materials and methods: We analyzed serum concentrations of sRAGE from samples of 168 children who progressed to type 1 diabetes and 43 children who became positive for at least 2 diabetes associated autoantibodies during prospective observation, but have not progressed to diabetes. We analyzed the sRAGE concentration before seroconversion, in the first autoantibody positive sample, and annually after that until the diagnosis of type 1 diabetes or end of follow up.

Results: The children who progressed to clinical type 1 diabetes were younger at seroconversion and at end of follow up than the non-progressors [2.04 (± 1.5) years vs. 3.04 (± 1.5) years, $p < 0.001$, and 5.91 (± 3.06) years vs. 9.67 (± 2.62) years, respectively (Mann-Whitney U-test)]. The progressors had similar or higher sRAGE than the non-progressors before seroconversion ($p = \text{NS}$) but subsequently lower levels [1183 (± 445) pg/ml vs. 1286 (± 490) pg/ml, $p < 0.001$ (t-test)]. The progressors had in the sample taken before seroconversion higher concentrations of sRAGE than in subsequent samples [mean sRAGE 1372 (± 573) pg/ml vs. 1183 (± 445) pg/ml, $p < 0.001$ (t-test)]. The difference remained significant even after adjusting for sampling age [$p < 0.001$ (linear regression)]. Concentrations of sRAGE were similar before and after seroconversion in the non-progressors [mean sRAGE 1290 (± 562) pg/ml vs. 1286 (± 490) pg/ml, $p = \text{NS}$ (t-test)]. There was a correlation between age and sRAGE concentrations among the non-progressors [$r = 0.13$, $p = 0.008$ (Spearman's rho)] but not in the children who eventually presented with type 1 diabetes. After seroconversion there was no significant variation in the sRAGE concentrations in the two groups.

Conclusion: Children who later develop type 1 diabetes have higher sRAGE concentrations before seroconversion than after the initiation of beta-cell autoimmunity. Our study suggests that non-progressors with persistent positivity to islet cell autoantibodies do not experience a similar decrease in sRAGE. As AGEs have been shown to be detrimental to beta cells, the drop in circulating sRAGE seen in temporal association with seroconversion may reflect failing protective mechanisms in the progressors.



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High risk vs low risk nondiabetic first degree relatives of patients with type 1 diabetes: differences in CXCR3+, CCR4+, CD25high T memory cells and chemokine levels

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Background and aims: Previously was suggested the prominent role of the chemokine receptors CXCR3 and CCR4, associated with Th1 and Th2 memory cell subsets respectively, together with impairments in T regulatory (T reg)

subset, in animal models of the initial phase of Type 1 diabetes (T1D). However, the relevance of the changes in CXCR3+ (Th1 associated), CCR4+ (Th2 associated) and CD25high subsets (T reg associated) of the T memory cells as well as in chemokine/cytokine levels, interferon- γ inducible chemokine (IP-10) (Th1 associated), thymus-and activation-regulated chemokine (TARC) (Th2 associated) and transforming growth factor β (TGF β) (Treg associated), for risk for T1D development, has not yet been clarified. Therefore, the aim of this study was to analyze (a) the percentage of CXCR3+, CCR4+, CD25high subsets of T memory cells and (b) chemokine/cytokine levels IP-10, TARC and TGF β , in peripheral blood of two groups, the high risk and the low risk group, of nondiabetic first-degree relatives (FDRs) of patients with T1D as well as in the group of healthy unrelated controls. The difference between the two groups of FDRs was based on presence/absence of glutamic acid decarboxylase (GADA) and tyrosine phosphatase insulinoma antigen-2 (IA-2) antibodies. Thus, in the study we included 17 high-risk nondiabetic FDRs (GADA+, IA-2+) (group A) and 34 low-risk nondiabetic FDRs (GADA-IA-2-) (group B) and 18 healthy unrelated controls (group C).

Materials and methods: T1D and glucose intolerance were excluded in the study by using WHO criteria. GADA and IA-2 levels were determined by ELISA. The percentages of CXCR3+, CCR4+ and CD25high T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flowcytometry. IP-10, TARC and TGF β were determined by ELISA.

Results: When the percentage of CXCR3+ T memory cells was analyzed, it was found to be higher in group A vs groups B and C (A: 64.98 \pm 5.19 vs B: 42.13 \pm 11.11; C: 53.09 \pm 6.29 %, A vs B: $p < 0.001$; A vs C: $p < 0.01$). In contrast, the percentage of CCR4+ T memory cells was significantly lower in group A vs groups B and C (A: 29.46 \pm 2.83 vs B 41.90 \pm 8.58; C: 40.90 \pm 7.24% $p < 0.001$). Simultaneously, the percentage of CD25high T memory cells was significantly lower in group A vs groups B and C (A: 0.13 \pm 0.05 vs B: 0.26 \pm 0.10 ; C: 0.26 \pm 0.06%, $p < 0.001$). On the other hand, IP-10 and TARC levels were significantly higher in group A vs B and C (A: 160.12 \pm 73.40; 438.83 \pm 120.62, B: 105.39 \pm 71.30; 312.04 \pm 151.14, C: 85.24 \pm 19.82; 236.88 \pm 89.19 pg/ml, respectively; A vs B $p < 0.05$, A vs C $p < 0.01$), while there was no difference in TGF β levels in groups A, B and C (A: 8883.17 \pm 3105.96, B: 7981.04 \pm 3889.51, C: 10064.44 \pm 681.99 pg/ml; A vs B vs C, $p = \text{NS}$).

Conclusion: Our results have demonstrated that high risk FDRs, showed higher levels of CXCR3+ T memory cells and IP-10, both associated with increased Th1 response, together with lower levels of CCR4+ T memory cells expressing Th2 response and CD25high T reg cells subsets, and without impairments in TGF β level. The results imply that the risk for developing T1D might be associated with the dominant influence of Th1 autoimmune response, together with diminished Th2 autoimmune response, while Treg response in high risk FDRs was impaired but still functionally preserved.

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Differential apoptosis in lymphocytes of patients with type 1 diabetes associated with relative expression of microRNA-146a

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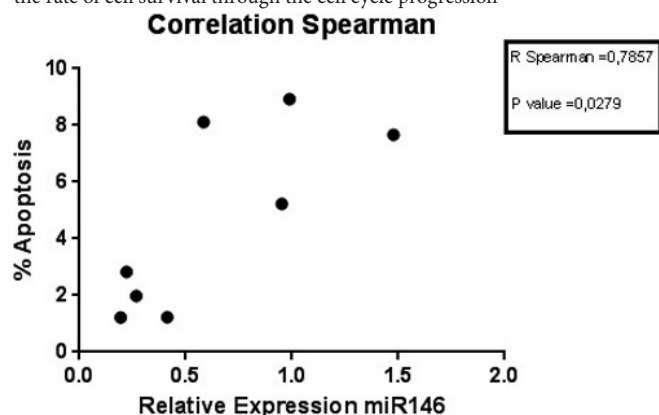
Background and aims: It is well established that type 1 diabetes (T1D) is an autoimmune disease. Controversial data exists regarding the differential control of the immune system in T1D patients compared to unaffected individuals. MicroRNAs (miRNAs) are involved in the control of gene expression (by negative regulation of gene expression at post-transcriptional level, by mediating translational repression or degradation of the mRNA targets). Their potential role in T cell activation and autoimmunity is controversial. We aim to elucidate relationship miR-146a in cell survival

Materials and methods: We investigated the expression profile of miRNAs in polymorphonuclear cells (PBMC) samples of 5 T1D patients (12-17 years old) and 5 healthy controls by means of qPCR. Cells were cultured in 96-well plates in RPMI-1640 with 10% FBS at 37 °C with 5% CO₂. The activation was performed using anti-CD3 1 μ g/ml for 8 hours. RNA extraction and cDNA were performed by standard techniques. miR-146, miR-155, miR-182 and miR-182. Real-time PCR expression on time zero and after 8 h of culture was measured. Additionally we quantified baseline lymphocyte apoptosis by flow cytometry using Annexin V.

Results: The comparison between diabetics and controls both in basal conditions and activation showed only miR146a statistically significant differences ($p < 0.0016$), miR155 ($p = 0.14$) and miR182 ($p = 0.95$). In relation to apoptosis, DM1 patients showed a lower rate of apoptosis (1.82%) and statistically different when compared with control subjects (7.49%), $p = 0.0286$. Finally,

the correlation between the percentage of apoptosis and expression of miR146a was $r = 0.786$ ($p < 0.0279$)

Conclusion: Our results indicate that the low expression of miR-146a may be associated with a decreased rate of cell apoptosis. Thus the activation window would be higher in these patients. Previous studies have shown that molecules such as IRAK and TRAF6 are targets of miR-146a, which work in a negative feedback system for activation of NF- κ B molecule that influences the rate of cell survival through the cell cycle progression



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Rapid assays for detection of type 1 diabetes-associated autoantibodies in organ donors

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Background and aims: Diabetes-associated autoantibodies are essential in the prediction of type 1 diabetes and in the classification of diabetes. Autoantibodies are also used to identify autoantibody-positive organ donors in large international studies (e.g. nPOD and PEVNET) which aim at uncovering pathogenic changes in pancreas and related organs in order to understand the mechanisms of beta-cell destruction. The current study set out to develop rapid assays for autoantibodies to glutamic acid decarboxylase 65 (GADA), islet antigen-2 (IA-2A) and zinc transporter 8 (ZnT8A) by modifications of both our in-house radiobindingimmuno assay (RBA) and a commercial ELISA (RSR Limited, United Kingdom).

Materials and methods: We have analyzed 100 diabetic children and non-diabetic siblings for IA-2A and GADA both with conventional and rapid assays. The RBA was modified into a rapid assay by incubating the labeled antigens and serum for one hour at RT instead of overnight at 4 °C. Immune complexes were bound to Protein-A Sepharose at 4 °C for one hour. The rapid assays were further optimized by separating serum and labeled antigen for 45 min at RT and immune complexes precipitated for 30 min at 4 °C. Further, antigen labels were made ready, standards and controls were pipetted on plates beforehand and all were kept at -80 °C. The RSR ELISAs were first performed according to the manufacturer's instructions. Then assays were modified by shortening the incubation times to 20 minutes and all incubations performed at RT. Six sera from the Finnish Pediatric Diabetes Register with varying autoantibody levels were selected and analyzed both with RIA and ELISA several times in different incubation conditions. For correlation analyses we also analyzed 17 diabetic and 20 non-diabetic siblings.

Results: The six sera from the Pediatric Diabetes register showed strong consistency for medium titers and negative responses, but RBA results close to cut-off values showed some discrepancies. ELISA results were more consistent. The analysis showed a strong correlation between the conventional and rapid RBA assays ($r=0.92$ for IA-2A and $r=0.89$ for GADA) with 1 hour incubations, ($r=0.912$ for IA-2A, $r=0.941$ for GADA and $r=0.862$ for ZnT8A) with 45 min incubation and 30 min precipitation. ELISAs performed according to the manufacturer's instructions and with the rapid assays correlated well ($r=0.96$ for IA-2A, $r=0.98$ for GADA and $r=0.87$ for ZnT8A).

Conclusion: Our results indicate, that all three (IA-2A, GADA and ZnT8A) RSR ELISA assays can be accomplished within 1 hour 45 minutes and the

results correlate well with results obtained according to the manufacture's instructions. All three RBA assays can be performed in 2 hours 15 minutes and the results obtained correlate well with conventional in-house assays and are in good concordance with the ELISA results.

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Combined appearance of autoantibodies against GAD, IA-2, insulin and ZnT8 in the islet autoantibody standardisation program 2013 proficiency workshop

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Background and aims: The Islet Autoantibody Standardization Program (IASP), a continuation of the Diabetes Antibody Standardization Program (DASP) of the Immunology of Diabetes Society, set up to evaluate and improve assays for type 1 diabetes (T1D)-associated autoantibodies (AAb). The IASP 2013 workshop aimed to assess the sensitivity/specificity and concordance of assays measuring AAb to GAD (GADA), IA-2 (IA-2A), insulin (IAA), and zinc transporter 8 (ZnT8A) in laboratories throughout the world. We assessed the combined detection of these four AAb in IASP serum samples considering differences in assay performance.

Materials and methods: Coded sera from 50 patients with newly diagnosed T1D and 90 healthy controls were analyzed in laboratories by 33 GADA assays, 31 IA-2A assays, 18 IAA assays and 21 ZnT8A assays. Sera were categorized according to the number of laboratories calling them positive using local assay thresholds, and further stratified between patients and controls by the number of positive AAb. Category 1 (C1) included sera that were found AAb positive by at least 90% of assays. Category 2 (C2) included C1-sera plus additional sera that were found AAb positive by at least 50% of assays. Category 3 (C3) included C2-sera plus sera that were found AAb positive by at least 25% of assays.

Results: Of 50 patient sera, 39 (78%) were included in C1, 42 (84%) in C2 and 45 (90%) in C3. None of the 90 control sera were included in C1 (0%), while 1 (1%) and 6 (7%) control sera were included in C2 and C3 respectively. The number of multiple AAb positive patient sera increased from 27 (54%) in C1, to 37 (74%) in C2, and 39 (78%) in C3 ($p=0.04$). One control serum in C2 was positive for multiple AAb. For all AAb specificities, C1-sera had significantly higher antibody titres than C2-sera ($p<0.0001$). Among the 45 AAb positive patient sera in C3, 18 (39%) had all four AAb, and another 13 (26%) had three positive AAb. The most frequently AAb combination was GADA plus IA-2A plus ZnT8A and positivity for all four AAb. Among single AAb positive sera, GADA were most frequently detected in controls ($n=3$).

Conclusion: We conclude that in the IASP 2013 proficiency workshop, laboratories with sensitive assays for GADA, IA-2A, IAA, and ZnT8A could detect AAb in almost all sera from newly diagnosed T1D patients, with multiple AAb in up to 90% of patient sera. Moreover, in these assays the number of patients with single AAb was reduced. AAb assays may infrequently detect positive signals against single but usually not multiple antigens in sera from controls. Thus the detection of different AAb is related to AAb level in both, patients and controls, and therefore dependent on assay sensitivity and threshold.

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Gadolinium chelate (Gd-DO3A-GAD), new contrast agent for non-invasive quantification of beta cell massK. Louchami^{1,2}, S. Liang², A. Rzajeva³, M. Aswendt⁴, A. Sener¹, U. Himmelreich²;¹Laboratory of EXperimental Hormonology, Université Libre de Bruxelles,²Biomedical MRI Unit/MoSAIC, Dept. Imaging & Pathology, KU Leuven,³Laboratory of Hormonology, Université Libre de Bruxelles, Belgium, ⁴Max Planck Institute for Neurological Research, Cologne, Germany.

Background and aims: The non-invasive imaging and quantification of pancreatic islet is considered a high-priority field of diabetes investigation. This work deals with the use of Gd-DO3A-GAD as new contrast agent in the perspective of pancreatic islets non-invasive imaging. The paramagnetic agent consists of Gd-DO3A as the Gd(III) chelate backbone. In its inactive state, long hydrocarbon side chains are anchored to one N atom of the Gd(III) chelate with glutamate moieties as head groups, which can enter the Gd(III) coordination sphere, thus limiting the water access to the paramagnetic core. Upon decarboxylation of the glutamate moieties due to the GAD (Glutamate decarboxylase) activity, there is an increase in the hydration sphere of the Gd(III) ion, leading to an increased T1 relaxivity. The present work, aims to perform the Glutamic acid decarboxylase characterization, the effect of Gd-DO3A on the insulin secretion evoked by D-glucose and the potential toxicity.

Materials and methods: The wistar rat pancreatic islets were obtained using the collagenase digestion method. Glutamate decarboxylase activity (14CO₂ production) was performed using the 14C-L-glutamic acid as substrate. The insulin secretion was measured by RIA (Radio Immuno-Assay) method. MRI experiments were performed using 9.4T Biospec small animal MRI system (Bruker, Ettlingen, Germany). Each phantom contains six tubes filled with 2% agarose in H₂O. Standard Bruker T1_map_RAREVTR sequence is used. (TE = 12.7ms, FOV = 5cm*5cm, Matrix size = 128*128).

Results: The Glutamate decarboxylase activity (14CO₂ production) was measured using the 14C-L-glutamic acid as substrate in the pancreas homogenates from normal vs diabetes (Streptozotocine) wistar rats demonstrate that the enzyme activity was 4 times fold higher in the normal animals. The time related L-glutamate decarboxylase activity in rat islets and brain homogenates after 120 min incubation reached respectively 45pmol/mg wet weight and 14pmol/1000islets. The present results demonstrate that the Gd-DO3A (50mM) fails to modulate the insulin secretion evoked by D-glucose at low (2.8 mM) or high (16.7 mM) of the hexose. Last, the Pancreatic islets (500), pancreatic islet cells (10.6) and INS1E (10.6) cells were incubated during 3hours in the presence of Gd-DO3A (50mM), phantom contains six tubes filled with 2% agarose in H₂O were prepared, and the MRI experiments performed using 9.4T Biospec small animal MRI system (Bruker, Ettlingen, Germany). The results show clearly that Gd-DO3A (inactive state) was activated probably due to GAD which catalyzes the conversion of glutamate into the major inhibitory neuron transmitter GABA.

Conclusion: L-glutamate decarboxylase enzyme, which catalyzes the conversion of glutamate seems also catalyze the Gd-DO3A. The enzyme activity is highest in the control than diabetic animals. Last, the Gd-DO3A-GAD fails to modulate the insulin secretion and didn't show any toxicity. The present results support the idea that Gd-DO3A-GAD could be used as a contrast agent for non-invasive quantification of beta cell mass.

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How to survive type 1 diabetes - the prolong studyV. Lyssenko^{1,2}, C. Möller¹, P. Storm², W. Poon², P. Vikman², L. Groop², P. Rossing¹, M. Eliasson³, B. Eliasson⁴, K. Brismar⁵, P.M. Nilsson²;¹Steno Diabetes Center A/S, Gentofte, Denmark,²Clinical Sciences, Lund University, Malmö, ³Department of Public Health and Clinical Medicine, Umeå University, ⁴University of Gothenburg,⁵Karolinska Institutet, Stockholm, Sweden.

Background and aims: To successfully prevent and treat diabetes complications, identification of protective mechanisms is required for a better mechanistic understanding of how the disease develops. PROLONG (PROtective Genes in Diabetic Complications and LONGevity) is a cross-sectional study of selected type 1 diabetic patients in Sweden and Denmark. The aim is to identify genetic markers, biomarkers and lifestyle factors, associated with protection from micro- or macrovascular diabetic complications in patients with long-standing type 1 diabetes.

Materials and methods: Patients with long diabetes duration (more than 30 years) with no major diabetic complications (i.e. nephropathy, proliferative retinopathy or laser treatment, neuropathy, myocardial infarction, stroke). As a comparator group we ascertain patients with early development of diabetic complications within 5-20 years of diabetes duration from the same geographical regions. DNA exome sequencing performed using Infinium HumanOmniExpressExome v1.1 DNA Analysis Kit. RNA sequencing performed using Illumina TruSeq Stranded mRNA Kit.

Results: Presently, 246 patients with type 1 diabetes are enrolled in the PROLONG study in Sweden (M/F %, 44/56, mean±SD, duration 38±12 yrs, HbA1c IFCC 64±14 mmol/mol). Based on hospital records at a diabetes centre in Denmark, we identified ~500 patients (M/F% 49/51, duration 40±9 yrs, HbA1c IFCC 54±10 mmol/mol) without major diabetic complications and 225 (M/F%, 50/50, duration 15±4 yrs, HbA1c 71.4±16.8 mmol/mol) who developed either macro-, or microvascular complications. Currently, the first DNA exome (n=96) and RNA sequencing analyses (n=96) have been completed on the Swedish PROLONG part. In line with previous observations in the Golden Years Project (UK), we observed that patients with long-standing diabetes without complications have higher HDL cholesterol (1.87±0.56 vs 1.40±0.45 mmol/l, p=1.8×10⁻³) and lower triglycerides 0.78±0.39 vs 1.12±0.46 mmol/l, p=2.9×10⁻³), and lower heart rate (74.4±10.5 vs. 83.2±14.7, p=0.001). Notably, we saw no differences in the levels of C-peptide (p=0.66), systolic (130±17 vs 130±22, p=0.86) or diastolic (89±9 vs. 92±11, p=0.18) blood pressure between individuals with or without complications. Furthermore, our preliminary analyses of the whole blood RNA expression pattern using Gorilla pathway analyses illustrated that sterol metabolism (DHCR24, SQLE, CYP51A1, HMGCS1, MSMD1 genes, etc) (cholesterol and sterol biosynthetic processes, p=9.9×10⁻⁷ and p=2.2×10⁻⁶; cholesterol and sterol metabolic processes, p=2.4×10⁻⁵ and p=4.6×10⁻⁵) was the top pathway associated with freedom from diabetic complications.

Conclusion: Our preliminary cross-sectional data suggest that patients with longstanding diabetes but free of major diabetic complications are characterised by a favourable lipid profile. Analyses are ongoing to verify, as well as dissect, the specific genomic markers that significantly differ in the sterol metabolism linked to this unique phenotype.

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Interleukin-15 and interleukin-6 concentrations in autoimmune diabetes

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Background and aims: Interleukin-15 and -6 (IL-15, IL-6) play a role in an inflammation, autoimmune, infectious and cancerous processes. Their increased concentrations were observed in psoriasis, asthma, multiple sclerosis and type 2 diabetes. However, the role of IL-15 and IL-6 in autoimmune diabetes pathogenesis is still unknown. The aim of our study was to evaluate of relationship between IL-15, IL-6 and CRP concentrations in persons with newly diagnosis of autoimmune diabetes in comparison to the first degree relatives of autoimmune diabetes and healthy controls.

Materials and methods: The group studied consisted of 54 persons with newly diagnosis of autoimmune diabetes (28 with Latent Autoimmune Diabetes in Adults (LADA) and 26 with type 1 diabetes (DM1)) and 70 healthy first degree relatives of patients with autoimmune diabetes and 60 healthy controls. GADA, IAA, IA-2A concentrations were measured by radioimmunoassays method. IL-15, IL-6 concentrations by ELISA method and CRP concentration by immunoturbidimetric method.

Results: We found significantly higher concentrations of IL-15 and CRP in the whole diabetes group in comparison to the group of relatives (p<0.001, respectively) and controls (p<0.001, respectively) and between relatives group and control group (p<0.001, respectively). IL-6 concentration was significantly higher in group of diabetes in comparison to the group of relatives and controls (p<0.001, respectively). We observed significantly lower IL-6 concentrations in relatives compared to the controls (p<0.001). In group of LADA and DM1 we found significantly higher IL-15 concentration in comparison to the group of relatives and controls (p<0.001, respectively). IL-15 was significantly higher in LADA compared to DM1 (p<0.001). We also observed significantly lower IL-6 concentration in relatives group in comparison to LADA and DM1 group (p=0.002, p<0.001). 31 relatives (44.3%) had positive concentration at least of one of the antibodies (Ab) against pancreatic islet. Relatives with Ab characterized significantly lower IL-6 concentration

compared to the relatives without Ab, control group and group of patients with LADA and DM1 ($p < 0.001$, respectively). IL-15 and CRP were significantly higher in the group of relatives with Ab in comparison to the group of relatives without Ab and control group ($p < 0.001$, respectively). In DM1 and LADA groups we observed positive correlation between IL-15 and CRP ($r = 0.22$, $p < 0.01$; $r = 0.25$, $p < 0.03$).

Conclusion: Significantly higher IL-15, IL-6 and CRP concentrations in the whole group of diabetes and in the subgroup of LADA and DM1 in comparison to the relatives and controls can prove increase risk of cardiovascular disease in the patients of newly diagnosis of autoimmune diabetes. However lower IL-6 concentration in the group of healthy relatives, especially with positive autoantibodies relatives can be marker delaying development of diabetes. IL-15 and IL-6 can be a good markers of risk of development autoimmune diabetes and IL-15 can be especially sensitivity marker for LADA diabetes.

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Beta cell autoantibody positivity is not useful to delineate type 1 diabetes in Southeast Asians

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Background and aims: There is a high prevalence of diabetes (DM) (11.3%) in Singapore. The estimated fourfold rise in DM prevalence in youth spells a grave outlook for the future burden of DM. Young onset diabetes presents a wide spectrum of aetiologies: type 1 and 2 diabetes (T1D & T2D), less well-defined subtypes e.g. latent autoimmune diabetes of adults (LADA) and monogenic DM. Each subtype portends a unique clinical course, optimum treatment modality and risk of transmission. In Asians, delineating these subtypes is challenging; the lower prevalence of β cell autoantibodies (Abs), lower age and lower BMI at onset of T2D compared to Caucasians increases this difficulty. Previous studies may not have assessed Abs to more recently discovered antigens e.g. Zinc Transporter 8 (ZnT8). **Aims:** 1. To describe the prevalence of β cell and thyroid Abs in a cohort with young onset DM (<45 years). 2. To look at the performance of β cell Abs in distinguishing between DM subtypes in a group of South-East Asian (SEA) patients.

Materials and methods: $n = 50$ (29F, 21M). SEA patients with young onset DM were recruited. Age at onset of DM, and BMI were recorded. Glutamic acid decarboxylase (GAD), ZnT8 and thyroid peroxidase (TPO) Abs were measured (IASP certified assays). Due to previous reports of low β cell Ab positivity in Asian T1D patients, this was not used as a criterion to classify DM subtype. Instead, patients were classified as having T2D if they were on oral anti-diabetic drugs (OAD) for at least 1 year before commencing insulin, were insulin independent or had no episodes of diabetes ketoacidosis (DKA), suggesting continued endogenous insulin production. The rest were classified as having T1D.

Results: The group was made up of 7 Malay (14%), 3 Indian (6%), and 40 Chinese (80%) patients. Based on the criteria above, 16 (32%) were classified as T1D, 34 (68%) as T2D. T2D patients had a higher BMI (mean 27.4 ± 5.1 vs 23.0 ± 3.5 , $p = 0.003$) and were older at DM onset (mean 26.0 ± 9.7 vs 17.3 ± 7.3 , $p = 0.003$). There was no significant difference in glycaemic control (T1D vs T2D: HbA1c 72 vs 76 mmol/mol). 6 of 16 (37.5%) T1D were positive for β cell Abs. Surprisingly, 5 of 34 (14.7%) T2D patients were positive for β cell Abs. 3 of these 5 were overweight/obese (BMI 26 – 32.1 kg/m²) by Asian standards. 1 of the 2 remaining β cell Ab positive T2D patients has maintained excellent glycaemic control (HbA1c 31 mmol/mol) on just one OAD without insulin since diagnosis 3 years ago. TPO positivity was equivalent between T2D and T1D (20.6% vs 18.8%). In total, β cell Abs was positive in 11 (22% of cohort). GAD Ab positivity ($n = 10$, 20%) was more common; $n = 4$ (8%) had raised ZnT8 Ab, 1 of whom was GAD Ab negative. There were no significant differences in BMI or age of DM onset between Ab positive or negative patients. 4 had a previous episode of DKA, just 1 of these had positive β cell Abs.

Conclusion: 1. In our cohort of SEA patients, a significant proportion has young onset non-insulin dependent DM. They are currently classified as having T2D, but may harbour rare variants of monogenic DM, and should be assessed further. 2. β cell Ab positivity was found in a significant proportion of T2D patients and its prevalence was low in T1D patients; it did not associate with insulin dependence, and did not delineate between clinically determined T1D and T2D. 3. Incorporating ZnT8 Ab to GAD Ab measurement did not offer much higher detection rates for β cell Ab positivity. This also further implies that autoimmunity may not be the major driving factor for β cell loss in Asian T1D patients.

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PS 009 Old genes, new phenotypes

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Genetic variation related to tolbutamide and GLP-1 stimulated insulin secretion converges functionally in protein network space: a DIRECT study

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Background and aims: Most proteins do not perform their biological function in isolation but rather in conjunction with other proteins in cellular machines or via interactions in metabolic or signaling pathways. This notion gives rise to genetic heterogeneity; the idea that genetic variants in different genes might cause identical or similar phenotypes, possibly by converging in pathway or network space. In this study we apply a systems biology data integration approach to investigate whether genetic variation related to tolbutamide or glucagon-like peptide-1 (GLP-1) stimulated insulin secretion response (ISR) functionally converges in protein network space.

Materials and methods: Genetic variations related to GLP-1 and tolbutamide ISR were identified in two genome-wide association studies (GWAS) in the DIRECT consortium; one on 130 individuals who underwent a modified hyperglycemic clamp with GLP-1 infusion, and one on 700 non-diabetic individuals who underwent a tolbutamide-modified IVGTT. Both GWAS were adjusted for age, sex, familial relationships and insulin sensitivity. GWAS p-values were collapsed to gene-associated p-values using MAGENTA and subsequently converted to z-scores. As tissue-specific protein-protein interaction (PPI) networks have previously been shown to outperform global networks in disease-gene prioritisation, a β -cell specific PPI network was created by pruning high confidence PPIs from InWeb 3.0 using published β -cell specific RNAseq data. Strongly connected components in the β -cell specific network were identified by ClusterONE. The degree of functional convergence of drug-response implicated genetic variation was assessed by a complex-based z-score, calculated by summing gene z-scores for all proteins in the complex for both drugs. The likelihood of observing a similar degree of functional convergence by chance was estimated by calculating the combined z-score for 10,000 degree preserving sets of random proteins.

Results: The systems biology approach integrating GWAS-data with a β -cell specific PPI network identified a connected component consisting of 34 proteins, which was significantly enriched for high-scoring genes associated with GLP-1- or tolbutamide ISR at $p \leq 1.0e-4$. This highly connected protein complex primarily consisted of guanine nucleotide binding proteins and members of the potassium inwardly-rectifying channel, both of which have previously been linked to beta-cell function. This result cannot be explained by high overlap between genes associated with tolbutamide- or GLP-1 ISR, as only two genes where among the top 100 genes for both gene-lists (*RAB-3GAP1* and *MYH13*).

Conclusion: We have shown that even though the 100 top-scoring genes related to tolbutamide- and GLP-1 stimulated ISR show limited direct overlap, a subset of those converges in a protein complex with a plausible and interesting biological role in insulin secretion.

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Intermediary diabetes mellitus (IDM): a new pathology between boundaries

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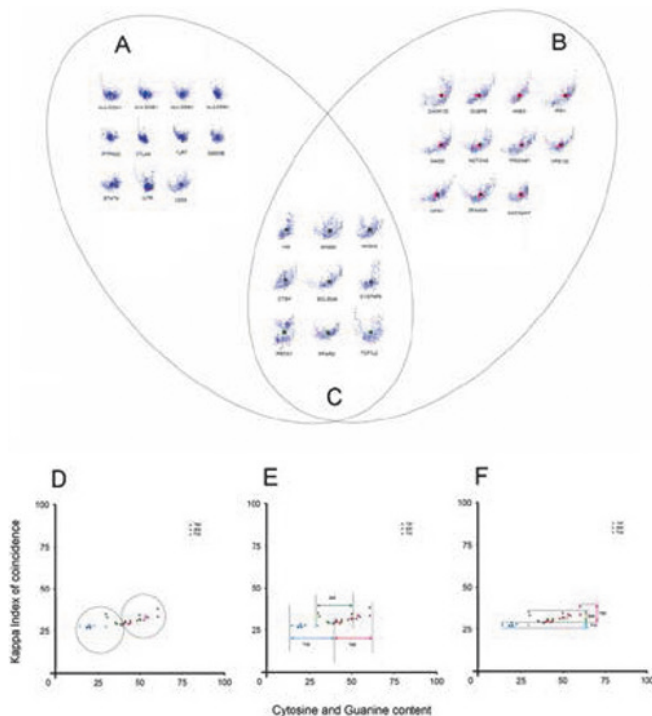
Background and aims: It is estimated that more than 30% of patients diagnosed with non-obesity-related type 2 diabetes may actually have what provisionally has been Latent Autoimmune Diabetes of Adults (LADA). Our study attempted to clarify the existence of this phenotype by using a new method for analyzing the promoters of genes associated with diabetes. Our

data shows that gene expression profiles could be the most important lead for detecting the primary cause of diabetes.

Materials and methods: In order to perform a generic analysis on gene expression profiles, we have used a total of 32 promoters of genes associated with classical T1D and T2D phenotypes. In order to measure the structural features of promoter sequences, we have used our original method of analysis based on DNA patterns (BMC Genomics 2012, 13:512). Thus, these DNA patterns can identify the transcription factors shared by genes associated with one or more phenotypes. Among the analyzed promoters of genes associated with classical T1D, we mention the HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DPB1, PTPN22, TLR7, CTLA4, GSDMB, STAT4, IL7R, C1QTNF6, CD55, CTSH, ERBB3 and INS gene promoters and for T2D the CAMK1D, DUSP9, HHEX, IRS1, MADD, NOTCH2, TP53INP1, VPS13C, WFS1, ZFAND6, HMGA2, PPARG, CDKN2AIP, PROX1 and TCF7L2 gene promoters (Figure A–C). In order to show the general relationship between the three phenotypes, a second distribution was made by using the center of weight of the patterns (Figure D–F).

Results: The promoters of genes associated with the two main phenotypes of diabetes contain different DNA patterns. T1D promoters exhibit image-based patterns which show that they are a part of a special class of promoters called „AT-based“ (Figure A). The promoters of genes associated with T2D exhibit patterns that show they are a part of a special class of promoters called „CG-based“ (Figure B). This separation of classes shows that genes associated with these two phenotypes rarely share transcription factors, therefore these genes can not be coexpressed. The third type of pattern is presented by a number of genes such as CD55, C1QTNF6, INS, ERBB3, HMGA2, CTSH, SLC30A8, CDKN2AIP, PROX1, PPARG, TCF7L2 which suggest a new phenotype, an Intermediary Diabetes Mellitus (IDM), (Figure C). The shape of these patterns indicate that genes associated with IDM can use transcription factors from both phenotypes, further indicating that IDM may contain the driver genes for triggering T1D and T2D.

Conclusion: Our current data showed that some promoters of genes associated with the two main phenotypes of diabetes seem to be associated to a third intermediary phenotype (IDM). These new data highlights the heterogeneity of different clinical phenotypes of diabetes.



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A common variant downstream of PCSK2 is associated with reduced tolbutamide stimulated insulin release: a DIRECT study

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Background and aims: We have previously shown that there is a high heritability of insulin secretion in response to a tolbutamide injection. The aim of this study was to perform a genome-wide association study to identify gene variants associating with tolbutamide stimulated insulin release.

Materials and methods: A total of 700 non-diabetic individuals, from two different cohorts, one family-based and one population-based, underwent a tolbutamide-modified frequently sampled IVGTT during which glucose was injected at 0 min and tolbutamide at 20 min. Measurements of plasma glucose, serum insulin and serum C-peptide were taken at 12 time points from fasting to 180 min and insulin secretion rate (ISR) and acute insulin response (AIR) were calculated. Genotyping was performed using the Illumina HumanCoreExome BeadChip. Association analyses adjusted for age, sex and BMI or age, sex and insulin sensitivity (Si) and separated by cohort were performed with EMMAX using the statistical package EPACTS, in order to handle relatedness in the samples. The results were then meta-analyzed using the GWAMA software.

Results: The strongest signal for reduced tolbutamide stimulated insulin release was seen downstream of the PCSK2 gene (AIR BMI adjusted: $\beta = -0.216$, $P = 1.6 \times 10^{-6}$, AIR Si adjusted: $\beta = -0.134$, $P = 4.2 \times 10^{-4}$), which has previously been associated with reduced glucose stimulated insulin secretion in humans.

Conclusion: Here we show that the PCSK2 locus, not only associates with reduced glucose stimulated insulin secretion as described previously, but also shows a strong signal for reduced tolbutamide stimulated insulin release. The signal is however not genome-wide significant and further replication is needed to confirm the association.

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Exome variation affects metformin treatment response

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Background and aims: Metformin is the first line treatment for type 2 diabetes. However the glycaemic response to metformin is highly variable between individuals. A recent study showed that the heritability of the absolute reduction in HbA1c was 34% and chromosome-wise heritability estimates suggested that individual genetic variants scattered across the genome account for the effect. The aim of our study was the identification of genetic factors that influence treatment response to metformin. This will help to optimize and personalize treatment in the future and it will give further insight into the working mechanism. In this study we focused on genetic variation in the exome, the part of the genome that is translated into protein because genetic variation in this region has a large a priori chance of being causal.

Materials and methods: Genetic variation was measured in 800 patients on metformin therapy from the Diabetes Care System ($n > 8000$), a large longitudinal cohort of Dutch type 2 diabetic patients, using the Illumina Exome chip. Twenty-five percent of the patients on metformin therapy did not reach the treatment target of an HbA1c ≤ 53 mmol/l (7%) within one year after initiation of therapy and we assessed if this treatment failure was associated with genetic variation using logistic regression. In addition we assessed the association with the absolute change in HbA1c after one year of treatment using linear regression. In both analyses baseline HbA1c, age, BMI, eGFR, and sex, metformin dose and metformin dual/mono therapy were included as covariates.

Results: Several genetic variants affected the response to metformin treatment in type 2 diabetes patients. The ten most significant gene variants gave a 1.7 to 8.5 fold reduced chance of reaching the treatment goal, an HbA1c level below 53 mmol/mol and the reduction in HbA1c ranged from 2 to 8

mmol/mol per risk allele (p 6.10 \times 10 $^{-6}$ to 3.10 \times 10 $^{-4}$). Most of these variants were low frequency variants and several were predicted to be damaging by SIFT/Polyphen2. However, due to the low frequency and power, replication in additional cohorts is necessary to reach genome wide significance. This replication is currently performed.

Conclusion: In this study we have identified a number of gene variants with relatively low frequency but a large effect on the metformin treatment response suggesting clinical usefulness in personalizing type 2 diabetes treatment. Furthermore, it identifies novel pathways modulating metformin response. However, replication in additional patients and cohorts is needed before definitive conclusions can be drawn.

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Insulin signalling genes exert a combined effect on all-cause mortality

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Background and aims: Type 2 diabetes (T2D) and cardiovascular (CV) disease are major factors increasing all-cause mortality. Both clinical entities recognize a common soil represented by insulin resistance (IR), which by itself also predicts all-cause mortality. IR is, at least partly, genetically determined. Thus, it is conceivable that genetic factors, which modulate IR, play also a role in modulating T2D, CV disease and all-cause mortality. In fact, we have previously reported the combined effect of single nucleotide polymorphisms (SNPs) perturbing insulin signaling (*ENPP1* K121Q, rs1044498; *IRS1* G972R, rs1801278; *TRIB3* Q84R, rs2295490) on IR and, as a likely consequence, T2D and major CV events. Based on these encouraging results, we investigated whether a combined effect of these 3 SNPs affects also all-cause mortality.

Materials and methods: We first studied a sample comprising 742 patients (i.e. discovery sample; 238 deaths/3,520 person-years; py). Replication was assessed in a second sample of 725 diabetic patients (i.e. replication sample; 129 deaths/5,495 py).

Results: In the discovery sample, weighted genetic risk score (GRS), based on each SNP's effect size, was associated with all-cause mortality (HR=1.12, 95% CI=1.03-1.23). After stratification according to low or high genetic load (GL) (i.e. 0-1 or > 2 risk alleles), patients with high GL ($n=123$) were at increased risk of all-cause mortality (HR=1.36, 95% CI=1.00-1.86), as compared to those with low GL ($n=619$). In the replication sample, HR (95% CI) for all-cause mortality was 1.06 (0.94-1.19) for GRS and 1.58 (1.06-2.35) for GL. In a pooled analysis (1,467 individuals; 367 deaths) both GRS and GL were associated with all-cause mortality HRs (95% CI)=1.11 (1.01-1.22) and 1.41 (1.10-1.80), respectively.

Conclusion: Our finding indicates that functional non-synonymous variants affecting insulin signaling exert a joint effect on all-cause mortality and is consistent with a pathogenic role of IR on life expectancy.

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Dissecting the genetic architecture of loci with established effects on multiple cardiometabolic phenotypes and type 2 diabetes

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Background and aims: Genome-wide association studies (GWAS) have identified hundreds of loci associated with Type 2 Diabetes (T2D) or other

cardiometabolic phenotypes, many of which overlap or map to the same genomic interval. Variants associated with multiple phenotypes, such as T2D, fasting insulin, triglycerides, HDL-cholesterol and body fat percentage influencing variants at IRS1, can provide insight into biology of correlated cardiometabolic phenotypes. However, the genetic architecture of these loci is frequently complex and needs further investigation.

Materials and methods: To disentangle association patterns of 630 associated SNPs (Dec 2012) from GWAS meta-analyses in Europeans for 19 quantitative phenotypes, T2D and hypertension, we defined sets of adjacent variants located less than 500kb apart and harboring 446 associated SNPs within 151 genomic regions (range=2-8 SNPs/region). We undertook approximate conditional analyses (ApCA) implemented in the GCTA tool to examine whether associations with multiple phenotypes within each region could be explained by LD.

Results: Across the 151 regions, we observed 14 (10%) loci in which the same SNP was associated with multiple phenotypes. Associations in 11 of these 14 loci were with epidemiologically highly correlated traits. Through ApCA, we identified 41 (27%) regions with multiple associated variants that underlie the same association signals, thus suggesting multi-phenotype effects. For 19 (13%) regions, the association with one phenotype partially explained the effect on another. Within 45 (30%) regions, multiple signals were explained by multiple non-related variants, whereas the remaining 32 (21%) regions showed complex architecture. Of the 44 regions associated with T2D, 15 contained the same association signal for other cardiometabolic phenotypes. Within 12 regions, including ANKRD55, SPRY2, DUSP8, PEPD and HNF4A, association with other phenotypes were not related to T2D variants. For 13 regions we observed complex architecture, while for the remaining 4 regions, the association with T2D partially explained the effect on another cardiometabolic phenotype.

Conclusion: Overall, a substantial number (87 or 58%) of cardiometabolic loci, of which 28 T2D loci, show potential pleiotropic effects on multiple phenotypes, which might contribute to their shared biology. Within other regions, distinct genetic effects or more complex architecture could underlie independent regulatory mechanisms.

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Genetic risk factors for diabetic complications in patients with type 2 diabetes from Ukraine

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Background and aims: Chronic hyperglycaemia is associated with increased risk of progression to macro (cardiovascular diseases) and microvascular complications (neuropathy, retinopathy and nephropathy). Recently, genome-wide association studies have identified a number of genetic loci for association with type 2 diabetes (T2D) and cardiometabolic traits. The effects of some of these genetic variants and the risk of diabetes progression to diabetes complications has been investigated in several populations, but has never been studied in Ukrainians, a population at high cardiovascular risk.

Materials and methods: We studied the association of a panel of 145 SNPs in loci previously reported to be associated with T2D/glycaemic traits ($n=75$), dyslipidemia ($n=11$), obesity ($n=8$), hypertension ($n=13$), cardiovascular diseases (CVD) ($n=21$) and microvascular complications ($n=17$) in approximately 3,500 subjects with T2D from the DOLCE study (M/F% 32/68, mean \pm SD, age-at-onset 53.5 \pm 10.6 years, BMI 31.4 \pm 5.6 kg/m², diabetes duration 7.1 \pm 7.4 years) (Diagnostic optimization and treatment of diabetes and its complications in the Chernihiv region). Effects of genetic loci were studied using logistic regression adjusted for sex and age-at-onset for macrovascular complications, and sex and diabetes duration for microvascular complications. The analyses were performed using R software, and genotyping was performed using Mass ARRAY iPLEX (Sequenom, San Diego, CA).

Results: We have replicated previous associations of GIPR rs10423928 (OR=1.29, $P=0.005$), WDR12 rs6725887 (OR=1.42, $P=0.005$) and MIA3 rs17465637 (OR=1.24, $P=0.04$) with CVD. VEGF rs2010963 (OR=1.28, $P=0.02$) previously reported to be associated with diabetic retinopathy (DR), was in our study associated with diabetic nephropathy (DN). The risk C-allele in TMEM26 rs1530440 (hypertension locus) was associated with increased

CVD risk (OR=1.18, P=0.04) and DN/DR (OR=1.4, P=0.01), while other two hypertension loci ATXN2 rs653178 (OR=0.87, P=0.03) and CYP17A1 rs11191548 (OR=0.75, P=0.03) with decreased CVD risk. Also, the ERBB4 rs7588550 (DN locus) was associated with increased risk for CVD (OR=1.79, P=0.01), while CVD loci PHACTR1 rs12526453 (OR=1.8, P=0.016) and PITX2 rs6843082 (OR=1.25, P=0.04) with increased risk for DR and DN.

Conclusion: We have replicated the previous association in GIPR, WDR12 and MIA3 for risk of CVD. Furthermore, we have demonstrated an association between risk alleles for hypertension and T2D/glycaemic traits loci and micro-/macrovascular complications. None of the studied BMI or dyslipidemia loci were significantly associated with complications in our study.

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Chemotactic cytokine receptor 5 (CCR5) gene promoter polymorphism (rs1799987) increases the risk of diabetic nephropathy in Asian type 2 diabetic patients

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Background and aims: To evaluate the association of CCR5/CCL5 variants with the risk of DN

Materials and methods: We conducted a systematic search of electronic databases (Pubmed, Embase and China National Knowledge Infrastructure) on three genetic variants (CCL5-403 G/A, CCL5-28 C/G, CCR5 59029G/A) and then 11 case-control studies involving 2512 DN cases and 2358 non DN control subjects were identified. The pooled odds ratio (OR) and 95% confidence interval (CI) were used to describe the strength of association with DN, the subgroup analysis was used to explore the heterogeneity bias among studies. Publication bias was tested by the Begg's and Egger's test.

Results: In the overall analysis, we found that CCR5 59029A-positive genotype (G/A or A/A) was an independent risk factor of DN (OR 1.69, 95% CI 1.13-2.55). Subgroup analysis demonstrated that the risk of CCR5 59029A-positive genotype was more significant among Asian patients with type 2 diabetic nephropathy (OR 2.08, 95% CI 1.68-2.57), but was nonsignificant in Caucasians or type 1 diabetes nephropathy (OR 1.23, 95% CI 0.52-2.92 for Caucasians; OR 0.82, 95% CI 0.56-1.20 for type 1 diabetes nephropathy). In addition, CCR5 59029A-positive genotype was associated with increased risk of albuminuria (OR 1.68, 95% CI 1.15-2.44 for microalbuminuria; OR 2.53, 95% CI 1.05-6.08 for macroalbuminuria). The CCL5 -403 G/A and CCL5-28 C/G gene polymorphism was not significantly associated with the risk of DN (OR 1.00, 95% CI 0.82-1.21 for CCL5-403 G/A; OR 1.02, 95% CI 0.79-1.33 for CCL5-28 C/G).

Conclusion: Our studies indicated that the CCR5 59029 A gene variant is a significant susceptibility factor for DN. Subtype of DM and ethnicity might contribute for the inconsistency present in studies. The CCL5 gene polymorphism (CCL5-403 G/A, CCL5-28 C/G) might not be a risk factor for DN.

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Indications for potential parent of origin effects within the *FTO* gene using long range phasing algorithms

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Background and aims: Genome-Wide Association Studies (GWAS) were successfully applied to discover associations with obesity. However, the GWAS design is based on unrelated individuals and clear inheritance information of phases is limited. Taking into account parent of origin may provide further insights into the genetic mechanisms contributing to obesity. We hypothesized there may be variants within the robustly replicated fat mass and obesity associated (*FTO*) gene that may confer different effect sizes for obesity depending on the transmission from father or mother.

Materials and methods: Genome-wide genotypes and pedigree information from the Sorbs population (N=525) were used. Phasing was done by applying long-range phasing and haplotype library imputation algorithm. Phased gen-

otypes among 525 individuals were generated by AlphaImpute. Subsequently, 22 SNPs within *FTO* introns 1 to 3 were selected and parent of origin specific association analyses were performed using PLINK: (i) standard association test, (ii) considering paternal and (iii) maternal alleles.

Results: We identified several SNPs conferring different P values depending on parental origin. Among them, rs1861868, rs1121980 and rs9939973 (all intron 1) show significantly different effect estimates beta (Student's t-Test; P<0.05). Of note, rs1121980 tags rs8050136 (r²=0.98) which was well replicated to be strongly associated with BMI.

Conclusion: Our results suggest that several *FTO* variants may underlie parent of origin effects modulating the risk of obesity. However, due to our limited sample size only large effects were detected. Further studies are warranted to investigate *FTO*.

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PARP-1 inhibitor, Olaparib, increases GLP-1 secretion and promotes both insulin secretion and TCF7L2 gene expression

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Background and aims: TCF7L2 is widely considered one of the most strongly type 2 diabetes (T2D) associated loci reported to date, but the pathophysiological mechanism by which variation within this gene modulates risk is poorly understood. We previously reported the specific protein factors that bind across the presumed causal variant at this locus, rs7903146, using oligo pulldown followed by mass spectrophotometry, with the most abundant of these being poly [ADP-ribose] polymerase type 1 (PARP-1) (Xia et al., 2014). PARP-1 has a potential role in the pathophysiology of diabetes, including evidence that PARP-1 knockout mice are protected from streptozotocin-induced diabetes. Proteomic studies have suggested that PARP-1 is also a component of the TCF7L2/beta-catenin complex. One important potential regulatory target of TCF7L2 is the pro-glucagon gene, which encodes a precursor of glucagon as well as other factors important for glucose homeostasis, including glucagon-like peptide-1 (GLP-1). We investigated if an existing PARP-1 inhibitor developed for oncologic indications, Olaparib, could have a GLP-1 agonist effect and thereby suggest regulation of PARP-1 mediated GLP-1 activity as one candidate mechanism for TCF7L2-related modulation of T2D risk.

Materials and methods: We used the human L-cell line, NCI-H716, for GLP-1 secretion assessment. Cells were pre-treated with 10uM Olaparib and after 48 hours the cells were transferred into PBS and incubated with or without 16mM glucose for 30 minutes, after which the suspensions were collected for GLP-1 ELISA. Next, we tested the effect of Olaparib on L-cell mediated augmentation of insulin secretion. To do this, we collected the medium from the treated NCI-H716 cells and used it to culture the human beta cell line, EndoC-BH1, for 30 minutes, after which we measured insulin by ELISA. Finally, we used real-time PCR to compare relative expression of TCF7L2 in NCI-H716 L-cells in the presence and absence of Olaparib.

Results: Treating NCI-H716 cells with glucose increased the release of GLP-1 into the medium as expected but, interestingly, Olaparib treatment also enhanced GLP-1 secretion significantly compared with the untreated group (51.1% average increase; $P < 0.05$). Furthermore, GLP-1 levels were further increased in the presence of the combination of glucose and Olaparib, compared with glucose or Olaparib alone (26.0% and 29.4% average increase, respectively; $P < 0.05$). The medium collected from the NCI-H716 cells treated with 10uM Olaparib increased insulin secretion over basal levels in EndoC-BH1 cells (15.2% average increase; $P < 0.05$). We also observed that TCF7L2 expression in HCI-H716 cells was highly statistically significantly increased (21.1% average increase; $P < 0.01$) by Olaparib.

Conclusion: Our results show that inhibition of PARP-1 in NCI-H716 cells increases GLP-1 levels and augments glucose-stimulated insulin secretion in EndoC-BH1 cells. PARP-1 inhibition also increases TCF7L2 gene expression in the NCI-H716 cells. Taken together, these observations suggest that TCF7L2 modulates T2D risk through its regulation of PARP-1 related GLP-1 activity. PARP-1 inhibition may represent an avenue for therapeutic intervention for T2D.

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Defective glucose homeostasis in mice inactivated selectively for Tcf7l2 in the adult beta cell with an Ins1-controlled Cre

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Background and aims: Single nucleotide polymorphisms in the TCF7L2 gene, including rs7903146, are associated with an elevated risk of type 2 dia-

betes in man. Previous studies of the effects of Tcf7l2 deletion in mice have provided conflicting results. In the present report we therefore inactivated Tcf7l2 specifically in pancreatic beta cells to investigate the cell-autonomous role for Tcf7l2 in these cells.

Materials and methods: To achieve highly selective deletion in the adult pancreatic beta cell, but not brain or other tissues, we crossed mice bearing floxed (exon 1) Tcf7l2 alleles to animals in which Cre recombinase was expressed from the Ins1 locus. Tcf7l2^{fl/fl}::Ins1Cre⁺, and littermate control mice were maintained on a C57BL/6 background and on either a normal or a high fat (60%; HFD; age 8 - 20 weeks) diet (Research Diet, New Brunswick, NJ, USA). Glucose tolerance was assessed by oral and intraperitoneal administration (1 g/kg body weight) following a 16 h fast, and insulin secretion *in vivo* measured after intraperitoneal injection of glucose (3 g/kg). Insulin secretion *in vitro* was measured from groups of six islets by radioimmunoassay (Millipore). Quantitative real-time PCR was performed on islet cDNA on a Fast 7500 device (ABI) running 7500 software (ABI) and with powerSYBR reagent (ABI). Changes in cytosolic calcium were assessed by Nipkow spinning disc confocal microscopy of whole islets loaded with fluo-2. Beta and alpha cell mass were assessed by optical projection tomography (19 µm resolution) of chemically-clarified pancreata double-stained for insulin and glucagon.

Results: Compared to littermate controls, Tcf7l2^{fl/fl}::Ins1Cre⁺ mice displayed impaired intraperitoneal glucose tolerance by 16 weeks (increase in AUC of 13.6 ± 2.8 %, $n=6$ mice per genotype, $p < 0.05$), and impaired oral glucose tolerance (increase in AUC of 10.6 ± 1.3 %, $n=6$, $p < 0.05$) from 8 weeks. Glucose intolerance was thus apparent earlier than in mice deleted for Tcf7l2 throughout the pancreas (Tcf7l2^{fl/fl}::Pdx1Cre⁺ mice; observed at 20 and 12 weeks when glucose was administered by the intraperitoneal and oral route, respectively). Islets of Langerhans isolated from Tcf7l2^{fl/fl}::Ins1Cre⁺ mice at 20 weeks displayed impaired glucose ($p < 0.05$) and GLP-1 ($p < 0.05$) stimulated insulin secretion, and decreased insulin and GLP-1 receptor gene expression ($p < 0.01$). Similarly, when maintained on a HFD, Tcf7l2^{fl/fl}::Ins1Cre⁺ mice displayed impaired glucose tolerance, and lower plasma insulin following an intraperitoneal glucose tolerance test, than littermate controls, and impaired GLP-1 stimulated insulin secretion ($p < 0.01$) and cytosolic calcium increases ($p < 0.03$). 20 week-old Tcf7l2^{fl/fl}::Ins1Cre⁺ mice that had been maintained on HFD for 12 weeks also displayed decreased ($n=4$, 31.7%, $p < 0.05$) beta cell mass, but normal alpha cell mass, compared to littermate controls.

Conclusion: These findings provide further support for the view that type 2 diabetes-associated Tcf7l2 variants may exert their effects, at least in part, through cell autonomous actions on the beta cell. These include impairments in Ca²⁺ signalling, and expansion in response to insulin resistance.

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RNA editing - an emerging role in diabetes?

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Background and aims: The genetic causes of T2D are still poorly understood. Previous studies have focused on discovery of genetic and epigenetic risk factors and gene-environment interactions. Less is known about post-transcriptional regulation of these risk-genes, RNA editing, in particular, which can alter genomically encoded information. Recent studies have shown that RNA editing may modulate multiple cellular functions. It was estimated that A-to-I RNA editing occurs at over a hundred million genomic sites. Hence, it is necessary to reveal the role of RNA editing events in the development of diabetes.

Materials and methods: To obtain putative A-to-I RNA editing events, we first built a prediction model based on the Darned database, containing a collection of editing sites from published sequencing data. Next, prediction was performed on common variants in the gene body of 104 risk genes for type-1 diabetes (T1D), type-2 diabetes (T2D), glucose traits and obesity. Finally, predicted sites of A-to-I RNA editing were checked in the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) consortium and an expression quantitative trait loci (eQTL) resource based on 5 311 human samples.

Results: A-to-I RNA editing events in Darned were mapped to 8 846 genes, including 46 genes related to diabetes, e.g., TCF7L2, FTO and ADCY5. GO enrichment analysis revealed that 1 540 out of these genes were enriched in metabolic processes ($p = 1.2 \times 10^{-13}$). There are 141 042 common variants (dbSNP build 138) located in the gene body of 104 diabetes related genes. We decided to keep only single nucleotide variants with reference/alternative allele as A/G, G/A, T/C and C/T. This resulted in 89 367 variants, out of which 25 399 were predicted as putative A-to-I RNA editing sites, using 20 flanking nu-

cleotides DNA sequences. Notably, 14 of these variants were found in risk loci for T1D, T2D, glucose traits and obesity (WFS1, CLEC16A, ZMIZ1, FUT2, CAPN10, TAGAP, KCNJ11, SH2B3, CTSN, HNF1B, PRKD2, SLC30A8 and KCNQ1), 2/197 were overlapped with DIAGRAM. The top nine loci ($p < 1.0 \times 10^{-30}$) were found located in TCF7L2, followed by six loci ($p < 1.0 \times 10^{-13}$) in CDKAL1. However, most (81%) loci did not show associations with diabetes. 485 putative sites of RNA editing were cis eQTL of 137 genes and 12 putative sites of RNA editing were trans eQTL of 51 genes (false-discovery rate of 0.50).

Conclusion: Our results suggest that RNA-DNA mismatches should be considered as an important factor in genetic studies of diabetes. The consequences of editing in risk alleles need to be further examined.

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Global Long Interspersed Nucleotide Element 1 (LINE-1) DNA methylation in a longitudinal cohort of type 2 diabetes mellitus patients

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Background and aims: Epigenetic mechanisms impact gene expression and could predispose individuals to a particular metabolic phenotype. Increased LINE-1 methylation has previously been associated with cardiovascular risk biomarkers in healthy individuals. Here we have investigated, for the first time, the relationship between global LINE-1 DNA methylation and cardio-metabolic parameters in Type 2 Diabetes Mellitus (T2DM) patients. This has been conducted using a well characterised longitudinal cohort from Salford, UK.

Materials and methods: Global LINE-1 DNA methylation was quantified by pyrosequencing in blood-derived DNA samples from 445 Caucasian T2DM patients using PyroMark Q96 CpG LINE-1 (Qiagen). The cohort consisted of Males 267 and Females 178; mean age 59.4yrs (males 58.7 years, females 60.4 years), with 7 years of longitudinal data. Global LINE-1 DNA methylation was analysed in relation to baseline anthropometric and biochemical measurements and their average changes per year over follow up, using multiple linear regression models adjusted for age, body mass index (BMI) and stratified by gender groups.

Results: Methylation at 4 CpG sites was quantified. The mean across the 4 sites was 75.1% (95% Confidence Interval (CI) 74.83% - 75.33%). There were no statistically significant methylation differences by gender (males 75.2%, females 74.90% $p=0.270$) or by age ($p=0.679$). Linear regression showed no significant associations with LINE-1 DNA methylation in the total cohort with; BMI [normalised beta coefficient 2.76 (95% CI -13.91 - 19.44, $p=0.745$)], total cholesterol [-1.59 (95% CI -4.02 - 0.83, $p=0.198$)], triglycerides [-2.77 (95% CI -6.76 - 1.22, $p=0.173$)], HDL-cholesterol [0.64 (95% CI -0.55 - 1.83), $p=0.293$] or LDL-cholesterol [-8.9 (95% CI -3.09 - 1.30, $p=0.425$)], or with systolic Blood Pressure (BP) [-22.51 (95% CI -66.75 - 21.28, $p=0.313$)] or diastolic BP [-17.81 (95% CI -44.00 - 21.28, $p=0.313$)]. Furthermore, there was no association with glycated haemoglobin (HbA1C) [-1.35 (95% CI -5.69 - 2.98, $p=0.540$)] at baseline or with average change per year in HbA1C over 7 years follow up [-0.004 (95% CI -0.014 - 0.006, $p=0.443$)] nor with levels of high sensitivity C-reactive protein (CRP) [13.57 (95% CI -21.36 - 48.52, $p=0.445$)]. However, stratification by gender revealed significant association of global LINE1 DNA methylation in women with LDL levels [-4.52 (95% CI -8.23 - 0.80, $p=0.017$)], with HDL: LDL cholesterol ratio [2.78 (95% CI 1.02 - 4.53, $p=0.002$)] and a trend also towards hypomethylation with total cholesterol [-3.76 (95% CI -7.87 - 0.34, $p=0.072$)].

Conclusion: Our findings indicate a gender related difference in global LINE1 DNA methylation with specific blood metabolic parameters related to cardiovascular risk in type 2 diabetes mellitus.

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Altered DNA methylation and differential expression of genes influencing metabolism and inflammation in adipose tissue from subjects with type 2 diabetes

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Background and aims: Genetics, epigenetics and environment may together affect the susceptibility for type 2 diabetes (T2D). Our aim was to dissect molecular mechanisms underlying T2D using genome-wide expression and DNA methylation data in adipose tissue from monozygotic twin pairs discordant for T2D and independent case-control cohorts.

Materials and methods: Genome-wide DNA methylation and mRNA expression were analysed using the Infinium HumanMethylation450 BeadChip from Illumina and GeneChip® Human Gene 1.0 ST arrays from Affymetrix, respectively.

Results: In adipose tissue from discordant twin pairs, we found that decreased expression of genes involved in oxidative phosphorylation, carbohydrate-, amino acid- and lipid metabolism, and increased expression of genes involved in inflammation and glycan degradation accompany T2D. The most differentially expressed genes included *ELOVL6*, *GYS2*, *FADS1*, *SPPI* (*OPN*), *CCL18* and *IL1RN*. We replicated these results in adipose tissue from an independent case-control cohort. Several candidate genes for obesity and T2D (e.g. *IRS1* and *VEGFA*) were differentially expressed in discordant twins. We found a heritable contribution to the genome-wide DNA methylation variability in twins. Differences in DNA methylation between monozygotic twin pairs discordant for T2D were subsequently modest. However, 15,627 sites, representing 7,046 genes including candidate genes for T2D and obesity showed differential DNA methylation in adipose tissue from unrelated subjects with T2D compared with controls. 1,410 of these sites did also show differential DNA methylation in the twins discordant for T2D. For the differentially methylated sites, the heritability estimate was 0.28. We also identified copy number variants in monozygotic twin pairs discordant for T2D.

Conclusion: Taken together, subjects with T2D exhibit multiple transcriptional and epigenetic changes in adipose tissue relevant to the development of the disease.

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Diet-induced obesity alters methylation of HOX transcription factors in mouse visceral adipose tissue

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Background and aims: Exposure to high-fat regimens induces epigenetic modifications which might shape the obesity phenotype. Indeed, recent work has revealed altered DNA methylation at several obesity-related genes in response to high-fat diets. However, the detailed methylome profiles determined by high-fat exposure remain to be elucidated. In the present study, we have implemented an unbiased genome-wide analysis of DNA methylation changes secondary to diet-induced obesity.

Materials and methods: Male C57BL/6J mice were fed either high-fat (HFD) or regular chow diets (ND) for 5 months. Methylated DNA immunoprecipitation combined with next generation sequencing (MeDIP-seq) were performed on DNA extracted from the visceral epididymal fat (VF) of the ND- and the HFD-fed mice. RT-qPCR and MeDIP-qPCR were then performed on the VF of these mice.

Results: Mice fed HFD gained weight (difference with the ND-fed mice significant at $p < 0.001$) and developed a significant decrease in their glucose tolerance (GTT, $p < 0.01$ vs. ND mice) and insulin sensitivity (ITT, $p < 0.001$ vs

ND mice). MeDIP-seq analysis identified differentially methylated regions (DMRs) covering with sufficient depth almost the entire genome and showed that, in the HFD-fed mice, the number of hypermethylated DMRs was higher than that of hypomethylated DMRs. Most DMRs were located within gene bodies. Based on subsequent gene ontology analysis of the MeDIP-seq data, differentially methylated genes (DMGs) were identified in the HFD-fed mice. Interestingly, a distinct set of genes belonging to the Homeobox (HOX) gene family was found to be epigenetically modulated by chronic exposure to HFD (Hoxa1, Hoxa3, Hoxa5, Hoxb3, Hoxb6, Hoxd1, Hoxa13). Hoxa5 was selected to validate the significance of this finding by measurement of its promoter methylation status and its gene expression. Results of the MeDIP-qPCR analysis ($p<0.05$) of Hoxa5 were consistent with those generated by the MeDIP-Seq studies. In addition, the Hoxa5 mRNA expression levels were significantly decreased in the VF of the HFD- compared to those of ND-fed mice ($p<0.05$), revealing an inverse correlation with its methylation state. These changes were accompanied by increased methyltransferase Dnmt3a and methyl-CpG binding domain protein Mbd3 mRNA expression levels ($p<0.05$), suggesting that the increased methylation of several genomic regions in the HFD-fed mice is mediated, at least in part, by increased expression of Mbd3 and Dnmt3a.

Conclusion: Our study on a genome-wide scale indicates that, in the mouse model, diet-induced obesity is associated with DNA methylation changes in VF. DMRs are enriched in genes regulating transcription, including the HOX transcription factors. This further finding suggests that an alteration of the methylation state of some members of this gene family and their impaired transcription may represent a mechanism by which a chronic high-fat feeding impacts on VF functions.

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Environmental exposures modulate Ankrd26 gene expression by inducing DNA methylation of its promoter

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Background and aims: Obesity and Type 2 Diabetes (T2D) represent major public health problems, making the need of achieving a better understanding of the pathogenesis of these disorders a priority. Similar to genetic polymorphisms, epigenetic modifications may alter transcriptional activity, impact on obesity and T2D risk and explain, in part, predisposition for these diseases. Based on MeDIPseq data, we have identified the Ankyrin repeat domain 26 (Ankrd26) gene as a target sensitive to environmental exposures. Previous work by us revealed that the Ankrd26 gene plays an important role in the control of food intake, fat mass and glucose tolerance in rodents. Indeed, mice with partial inactivation of this gene develop marked hyperphagia, severe obesity and diabetes. We aimed to investigate whether obesity in vivo or elevated free fatty acids (FFAs) in vitro may impact on Ankrd26 gene transcription by causing epigenetic modifications.

Materials and methods: Gene expression was evaluated by Real-time PCR analysis in the epididymal white adipose tissue (WAT), the tibialis skeletal muscle (SM) and liver (L) from diet-induced obese (DIO) C57Bl/6 mice and in 3T3-L1 adipocytes treated for 24, 48 and 96 hours with palmitate (PAL; 0.25 mM) or oleate (OLE; 0.25 mM). DNA methylation of Ankrd26 promoter (CGI: from -726 to -226 bp) was investigated by MeDIP analysis in WAT from DIO mice and in PAL treated adipocytes.

Results: Ankrd26 mRNA expression is decreased in WAT and SM but not in L from DIO mice (WAT: C 0.509 ± 0.011 AU vs. DIO 0.483 ± 0.008 AU, $p<0.001$; SM: C 0.578 ± 0.019 AU vs. DIO 0.558 ± 0.011 AU, $p<0.01$; L: C 0.492 ± 0.023 AU vs. DIO 0.500 ± 0.028 AU). In addition, PAL treatment for 96 h induces a 20% decrease of Ankrd26 mRNA levels in mature adipocytes, as well (C 1.00 ± 0.00 REU, PAL24h 0.95 ± 0.09 REU, PAL48h 1.05 ± 0.08 REU, PAL96h 0.82 ± 0.04 , $p<0.001$). No significant effects on Ankrd26 expression are observed when cells are treated with OLE. MeDIP analysis shows a 3-fold increased immunoprecipitation of a DNA fragment covering a portion of Ankrd26 CGI both in WAT from DIO mice and in PAL-treated adipocytes. Interestingly, PAL treatment induces a 20% increase of the de novo DNA methyl transferase (DNMT) 3b mRNA expression (C 1.00 ± 0.00 REU, PAL 96h 1.19 ± 0.03 REU, $p<0.01$).

Conclusion: Hyper-methylation of Ankrd26 promoter occurs both in vivo in WAT from DIO mice and in vitro in PAL-treated adipocytes, and is inversely correlated to its mRNA expression. This suggests that environmental exposures impact Ankrd26 transcription by inducing epigenetic changes.

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DNA hyper-methylation of the zinc transport (SLC30A8) gene is associated with type 2 diabetes in a Malay population

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Background and aims: The solute carrier family 30 member 8 (SLC30A8) gene codes for a zinc efflux transporter related to insulin secretion in pancreatic beta-cells. Recent genetic studies have demonstrated that the common alleles of single nucleotide polymorphisms (SNPs) including rs13266634 (C/T, Arg276Trp) and rs11558471 (A/G) in the SLC30A8 gene confer the risk susceptibility to type 2 diabetes (T2D). But the question regarding whether this gene has epigenetic effects in T2D is still remained.

Materials and methods: To address this question, we first replicated genetic association study of the SLC30A8 gene in 992 Malay subjects with normal glucose tolerance (NGT) and T2D. Genotyping experiments were conducted with TaqMan allelic discrimination. Analyses based upon genotypes and haplotypes constructed with these two SNPs rs13266634 and rs11558471 provided compelling evidence of association with T2D in this Malay population. We then performed DNA methylation analysis of four CpG sites in the SLC30A8 gene promoter with bisulfite pyrosequencing.

Results: The A allele of SNP rs11558471 (A/G) and the common haplotype C-A constructed by the major alleles of this polymorphism and rs13266634 were found to be associated with T2D ($P=0.002$, $OR=1.334$, $95\%CI=1.110-1.602$; $P=0.008$, $OR=1.233$, $95\%CI=1.055-1.442$). The averaged DNA methylation levels in the SLC30A8 gene promoter in the Malay cohort were high (~78.5%). DNA methylation levels in the SLC30A8 gene were increased in T2D patients compared with NGT subjects (79.9%, $95\%CI=79.2-80.5\%$ vs. 77.1%, $95\%CI=75.4-78.6\%$, $P=0.001$).

Conclusion: The present study demonstrates for the first time that DNA hyper-methylation of the SLC30A8 gene is associated with T2D in a Malay population and suggests that this gene has epigenetic effects in T2D.

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Visceral fat tissue content and HOMA-IR better predicts type 2 diabetes risk than genetic risk score based on 70 SNPs identified by GWAS for type 2 diabetes and/or obesity

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Background and aims: The prevalence of obesity is growing rapidly effecting the large increase in type 2 diabetes (T2DM) incidence. To stop this epidemic trend it is important to find the method for early prediction of type 2 diabetes risk and motivation to begin an early lifestyle intervention. For the personal T2DM risk evaluation several factors, such as age, sex, ethnicity family history are well documented and the data concerning the role of genetic factors and gene-environment interaction has been recently accumulated. A precise interaction of these risk factors is, however, a complex process that varies both within and across populations. The aim of our study was to evaluate the role of clinical parameters and recently discovered genetic variants as a markers for type 2 diabetes for early prediction.

Materials and methods: Study group consisted of 945 Caucasian origin volunteers without previously known dysglycaemia collected between 2009 and 2012 for the prospective 1000PLUS cohort study (463 women and 482 men, aged 18-65 years old, mean age 40.4 ± 0.8 yrs). Among the study population 634 subjects were overweight/obese and 311 people had BMI<25. In all subjects demographic data, anthropometric measurements have been recorded;

blood samples at fasting for metabolic (glucose and insulin) and genetic analyses were collected. The oral glucose tolerance test (OGTT) have been performed and type 2 diabetes was diagnosed based on WHO criteria. Body composition: percentage of total body fat, visceral (VAT) and subcutaneous adipose tissue (SAT), VAT/SAT ratio were also analyzed by bio-impedance method. Genetic risk score (GRS) for each participant was calculated using previously identified 70 loci by weighting the number of risk alleles corresponding to a particular locus by its effect size (β -estimate) on diabetes risk and summing these values. The studied genotypes distributions were in Hardy-Weinberg equilibrium ($p>0.05$).

Results: In the logistic regression analysis the risk of type 2 diabetes development was significantly associated with age [OR=1.2 (1.1–1.4), $p=2.8\times 10^{-6}$], HOMA-IR [OR=1.9 (1.4–2.6), $p=1.9\times 10^{-5}$], VAT/SAT ratio [OR=11.4 (2.0–91.7), $p=0.01$] and GRS [OR=1.13 (1.05–1.2), $p=2\times 10^{-5}$] but not with BMI and gender. When particular SNPs were introduced into the regression model instead of GRS the strongest association with type 2 diabetes risk was found for rs11558471 SLC30A8 ($p=0.03$), rs226000 PRRC2A ($p=0.018$), rs11708067 ADCY5 ($p=0.008$), rs2844479 AIF1 ($p=0.02$), rs10938397 GN-PDA2 ($p=0.001$), rs7647305 SFRS10 ($p=0.01$), rs174550 FAD1 ($p=0.016$), rs10946398 CDKAL1 ($p<0.04$), rs780094 GCKR ($p=0.03$), rs340874 PROX1 ($p=0.044$) assuming the additive model.

Conclusion: We found that visceral fat tissue content and HOMA-IR better predict type 2 diabetes risk than the genetic risk score (based on 70 SNPs identified by GWAS for type 2 diabetes and/or obesity). However both of them, the clinical and genetic factors, allow for better individual prediction of type 2 diabetes.

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PS 011 New and old genes for monogenic diabetes

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Mutation in APPL1 gene may contribute to familial diabetes mellitus

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Background and aims: Familial, apparently autosomal dominant, early-onset forms of diabetes mellitus of the adulthood that are negative for mutations in known MODY genes have been repeatedly described. Except that for their age of onset, these forms of diabetes resemble classical type 2 diabetes (T2D), thereby suggesting that they are due to mutations in genes modulating insulin sensitivity and/or the fine tuning of insulin secretion rather than in genes playing a central role in insulin synthesis/ secretion, as is the case for MODY. The aim of this study was to identify genetic causes of these forms of diabetes. **Materials and methods:** To accomplish this aim, whole exome sequencing was carried out in probands from 59 families from Italy and the US. New identified mutations were studied by transfection in HepG2 cells followed by evaluation of mRNA and protein expression levels along with insulin-stimulated Akt2-ser⁴⁷³ and GSK3 β -ser⁹ phosphorylation.

Results: Two mutations in the APPL1 gene (a L552X stop-codon and a D94N missense mutation), showing segregation with diabetes, were found in two different families. Neither mutation had been previously reported in publicly available databases. APPL1 binds to Akt2 and positively modulates insulin-mediated Akt2 activation and downstream signaling, which is involved in both insulin action and secretion. Accordingly, APPL1 ablation causes insulin resistance and impaired glucose homeostasis in mice. X552 totally abolished APPL1 protein (but not mRNA) expression, consistent with its being a stop-codon mutation. N94 APPL1 was expressed at the same level as WT APPL1; however, it caused significant reduction in the enhancement of insulin-stimulated of Akt2 and GSK3 β phosphorylation observed after WT APPL1 transfection as compared to un-transfected cells, therefore behaving as a loss of function mutation. Both mutations were absent in 2970 T2D patients and in 1639 control subjects.

Conclusion: This is the first evidence of APPL1 mutations contributing to familial forms of diabetes mellitus of the adulthood and reaffirms the critical role of this gene in glucose homeostasis.

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Enhanced transcriptional activity of the K392R mutant form of STAT3 found in a patient with early onset diabetes is not fully dependent on phosphorylation of Tyrosine-705

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Background and aims: We have recently reported a series of 4 novel activating germline mutations in STAT3 among patients exhibiting an early onset poly autoimmune syndrome including autoimmune diabetes. In the current study we have explored the molecular basis of the enhanced activity of one of these activating mutants, K392R.

Materials and methods: Wildtype (WT) and mutant (K392R) STAT3 were generated and transfected transiently into HEK293 cells. In addition, stable INS-1E cells were also created expressing these constructs under the control of a tetracycline inducible promoter. Transcriptional activity was assessed using a STAT3-responsive reporter construct while total and phosphorylated STAT3 were detected by Western blotting.

Results: Transfection of WT and mutant (K392R) STAT3 into HEK293 cells resulted in equal levels of protein expression but differing levels of transcriptional activation. Notably, the K392R mutant dramatically increased STAT3 reporter activity relative to the WT (by 37.1 ± 4.7 fold; $p < 0.01$). Enhanced reporter activity was also observed in INS-1E cells expressing K392R although the absolute increase was smaller than in HEK293 cells (4.0 ± 0.4 fold; $p < 0.01$). Classically, STAT3 activity is regulated mainly by phosphorylation at Tyr 705 but the extent of phosphorylation of this residue was only modestly increased in the mutant under unstimulated conditions (3.1 ± 0.6 fold vs WT; $p < 0.05$) despite its markedly enhanced transcriptional activity. Addition of IL-6 (20 ng/ml) resulted in an increase in STAT3 activity in cells expressing either WT or K392R, although the increase was much greater for the mutant (a further 7.3 ± 1.4 fold enhancement above that seen in WT cells stimulated with IL-6; $p < 0.05$). IL-6 also further increased the extent of phosphorylation of Tyr 705 in both WT STAT3 and in the K392R mutant. The increased STAT3 activity of K392R was reduced, but not abolished, by pharmacological inhibition of the upstream kinase Jak which promotes phosphorylation of Tyr 705 (fold change from WT: K392R - 30.7 ± 8.4 , K392R + Jak inhibitor - 6.0 ± 0.8 $p < 0.05$) suggesting that the enhanced activity is not dependent fully on Tyr 705 phosphorylation. To further assess the importance of this residue, Tyr 705 was replaced by phenylalanine (Y705F) to create a double mutant (K392R/Y705F). This was less active than K392R in reporter assays but, despite the loss of Tyr 705, it still retained enhanced transcriptional activity in both the absence or presence of IL-6 ($p < 0.01$).

Conclusion: The constitutively active K392R germline mutation of STAT3 found in a patient with poly autoimmune syndrome and early onset diabetes, induces a strong transcriptional activation which may contribute to the autoimmunity. The mechanism of activation is atypical and does not depend solely on increased phosphorylation of Tyr 705.

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The phenotypic severity of homozygous GCK mutations causing neonatal or adolescent-onset diabetes is mediated through thermostability in addition to enzyme activity

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Background and aims: Mutations in the glucokinase (GCK) gene cause a spectrum of glycaemic disorders. Homozygous loss-of-function mutations are rare and cause permanent neonatal diabetes (GCK-PNDM) requiring lifelong insulin treatment. Heterozygous loss-of-function mutations cause mild fasting hyperglycaemia irrespective of mutation severity due to compensation from the unaffected allele.

Materials and methods: We determined the relationship between in vitro mutation severity and clinical phenotype in a large international case series of patients with homozygous GCK mutations.

Results: Clinical characteristics for 30 patients with diabetes due to homozygous GCK mutations (19 unique mutations, including 16 missense) were compiled and assigned a clinical severity grade (CSG) based on birth weight and age-at-diagnosis. The majority (28/30) of subjects were diagnosed by 9 months of age, with the remaining two in adolescence. These are the first two cases of a homozygous GCK mutation diagnosed outside the neonatal period. Recombinant mutant GCK proteins were analysed for kinetic and thermostability characteristics and assigned a relative activity index (RAI) or relative stability index (RSI) value. Six of the 16 missense mutations exhibited severe kinetic defects ($RAI \leq 0.01$). There was no correlation between CSG and RAI ($r^2 = 0.02$, $p = 0.65$), indicating that kinetics alone did not explain the phenotype. Eighty percent of the remaining mutations showed reduced thermostability, the exceptions being the two adolescent-onset mutations which exhibited increased thermostability. Comparison of CSG with RSI detected a highly significant correlation ($r^2 = 0.63$, $p = 0.006$).

Conclusion: Within the largest case series to date we demonstrate that homozygous GCK mutations can cause adolescent-onset diabetes. Protein instability is the major determinant of mutation severity.

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Cystatin C-based GFR estimate is higher than the creatinine-based GFR in HNF1A MODY and GCK MODY: results of a replication study

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Background and aims: Cystatin C is a marker of glomerular filtration rate (GFR). Its level is dependent on the concentration of CRP (C-Reactive Protein), which is decreased in diabetic patients with an HNF1A-MODY. We examined previously the cystatin C as a biomarker for the HNF1A-MODY with inconsistent results and showed that the cystatin C-based GFR estimate is higher than the creatinine-based one. Currently, we aimed to perform a replication study using a new cohort of HNF1A-MODY patients and, additionally, glucokinase (GCK) MODY subjects.

Materials and methods: The study included 63 patients with HNF1A-MODY, 70 with GCK-MODY, 44 type 1 diabetes (T1DM), 47 type 2 diabetes (T2DM) patients and 61 subjects without diabetes. Creatinine was measured using Roche P800 analyzer (Roche Diagnostics, Burgess Hill, UK). The level of cystatin C and CRP was determined using enzyme immunoassay. GFR was calculated from the following: the creatinine with the CKD - EPI formula, and from cystatin C with the 4-variable formula. Statistical analysis to determine the difference between two (t- student) and several groups (ANOVA with post-hoc tests) was used. If necessary, non-parametric tests were utilized as equivalents. Differences in the level of cystatin C between groups (GCK-MODY, HNF1A-MODY, control, T2DM, T1DM) were adjusted for gender, age, BMI and estimated GFR (creatinine) and determined by analysis of covariance (ANCOVA). Statistical analyses were performed using STATISTICA software 10.0.

Results: Cystatin C level in post-hoc analysis (Scheffe test) was significantly lower ($p < 0.0001$) in the control group ($0.73 \text{ mg/l} \pm 0.14$), HNF1A-MODY ($0.74 \text{ mg/l} \pm 0.21$) and GCK-MODY ($0.71 \text{ mg/l} \pm 0.15$) when compared to patients with T1DM ($0.90 \text{ mg/l} \pm 0.15$) and T2DM ($0.96 \text{ mg/l} \pm 0.24$). The differences between the groups became borderline after adjustment for the gender, BMI, age and eGFR. Comparison of GFR estimated from the creatinine level (CKD-EPI) with GFR from cystatin C (CysC eGFR) has shown no difference in T2DM and control group ($p = 0.6938$). T1DM patients had significantly higher CKD-EPI than CysC eGFR ($p = 0.0002$). An inverse relationship was observed in MODY patients: CysC eGFR was significantly higher compared to the CKD-EPI in both GCK ($p = 0.031$) and HNF1A ($p = 0.0143$) MODY patients.

Conclusion: We confirmed that GFR value estimated from cystatin C level in MODY patients is significantly higher compared to GFR estimated from creatinine. Interestingly, a similar finding was identified in GCK-MODY. However, Cystatin C was not a good biomarker to differentiate between various types of diabetes.

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Intima - media thickness and endothelial dysfunction in GCK and HNF1A MODY patients

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Background and aims: Mutations in the GCK (glucokinase) gene are, along with the HNF1A gene mutations, the most frequent cause of MODY (maturity onset diabetes of the young). Heterozygous loss-of-function GCK mutations result in a moderate fasting hyperglycemia. The GCK-MODY patients are usually free from microvascular complications; however, little is known about atherosclerosis and intermediate related phenotypes. We aimed to examine intima-media thickness (IMT) and endothelial function in GCK-MODY and HNF1A-MODY compared with controls.

Materials and methods: 66 GCK-MODY, 50 HNF1A-MODY patients and 54 non-diabetic controls were examined. Carotid artery IMT and brachial artery FMD and NMD (nitroglycerin mediated dilatation) were assessed by ultrasonography. These parameters were compared with test for difference between two groups (t-test or U Mann Whitney test) or three groups (one way analysis of variance ANOVA or the Kruskal-Wallis test with post hoc test).

Results: The average maximum IMT was different among three groups - $0.71 \pm 0.17 \text{ mm}$ in GCK-MODY, 0.77 ± 0.15 in HNF1A-MODY, and 0.71 ± 0.15

in the controls ($p=0.0041$). In post hoc analysis maximum IMT for GCK-MODY was statistically different from HNF1a MODY ($p=0.0371$) Mean IMT were as follow - 0.62 ± 0.14 mm in GCK-MODY, 0.68 ± 0.13 in HNF1a-MODY and 0.63 ± 0.13 in controls ($p=0.0077$). Post hoc analysis showed a difference between GCK-MODY and HNF1a-MODY ($p=0.0108$). The mean FMD% was $11.0\pm4.5\%$ in GCK-MODY, $10.1\pm4.9\%$ in HNF1a-MODY and $13.9\pm4.8\%$ in the controls ($p=0.0001$). The differences between CK-MODY and HNF1a-MODY vs. controls were significant ($p=0.0045$, $p=0.0001$, respectively). I was similar in all three groups - 24.1 ± 4.5 , 24.1 ± 3.7 and 24.2 ± 3.6 in GCK-MODY, HNF1a-MODY and controls, respectively ($p=0.7876$). Patients were diagnosed with diabetes at similar age (GCK-MODY: 25.1 ± 13.5 yrs vs. HNF1a-MODY: 26.6 ± 11.6 , $=0.4669$). Glycemic control was similar in diabetic groups as in the GCK-MODY group the mean HbA_{1c} was $6.4\%\pm0.7$, while in HNF1a-MODY patients it reached 6.7 ± 1.4 ($p=0.6936$).

Conclusion: Both examined MODY groups showed evidence of early atherosclerosis or endothelial dysfunction. Mild hyperglycemia in the GCK-MODY seems to have a fragile impact on the occurrence of intermediate atherosclerotic phenotypes.

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Clinical features of MODY-HNF1a in children and adolescents with obesity

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Background and aims: to study clinical features of MODY-HNF1a in children and adolescents with obesity.

Materials and methods: 18 patients who had heterozygous mutation in HNF1a gene were divided into 2 groups: group 1 - the patients with SDS BMI ≥ 2 ($n=6$; 33.3%); group 2 - the patients with SDS BMI < 2 ($n=12$; 66.6%). We measured fasting and stimulated glucose, C-peptide, insulin levels (OGTT). The data is presented as medians (25; 75 percentile), Mann-Whitney U-test was used to compare medians.

Results: Age at diagnosis of diabetes: in group 1 was 10.4 years (9.5;14.4), in group 2 was 12.8 years (10.9;15.4), $p<0.05$. Diagnosis of diabetes in group 1: 33.3% of patient were investigated due to suspected diabetes cases because of family history, 33.3% of patient were investigated due to obesity, 33.3% of patient had the clinical features of diabetes; diagnosis of diabetes in group 2: 16.7% of patient were investigated due to suspected diabetes cases because of family history, 75% of cases were diagnosed occasionally, 8.3% of patient had the clinical features of diabetes. At the diagnosis HbA_{1c} : in group 1 was 7.3% (6.8; 7.9); in group 2 was 6.5% (6.3; 8); ($p>0.05$). 100% patients in group 1 and 91.6% patients in group 2 had family history of diabetes; one of the parents had insulin dependent diabetes mellitus (66.6% and 25%, respectively group 1 and group 2), non insulin dependent diabetes mellitus (16.65% and 58.3%, respectively group 1 and group 2), gestational diabetes mellitus (16.65% and 8.3%, respectively group 1 and group 2). Disease duration was 3 years (1.1; 4.5), when diagnosis MODY-HNF1a was confirmed. We identified the novel mutations: D45fs, V119G, R229X, S249Stop. The most common Pro291fsinsC mutation was identified in 5 cases (27.7%). Clinical data is presented in Table 1. Patients in group 1 had significantly higher HbA_{1c} and fasting serum C-peptide. Two patients in group 1 were insulin resistant. Patients in groups 1 and 2 were treated with sulfonylurea (50% of all patients) and metformin (50% and 8%, respectively). 42% patients in group 2 were treated with diet only.

Conclusion: MODY-HNF1a may be combined with obesity and in some cases with insulin resistance in children and adolescents. Obesity is a risk factor for early manifestation and more severe diabetes. Patients in group 1 had higher C-peptide secretion. DNA testing of patient with mild diabetes, obesity and family history of diabetes could lead to diagnosis of new cases of MODY-HNF1a.

Table 1. Clinical data of patients with MODY-HNF1a

Clinical data	Group 1	Group 2	p
HbA1c, %	7.8 (7.4; 7.9)	6.3 (6; 7)	$p<0.01$
C-peptide 0 min, ng/ml	2.2 (1.9; 2.6)	1.3 (1.1; 1.4)	$p<0.01$
C-peptide 60 min, ng/ml	4.3 (3.5; 5.2)	3.6 (3.3; 4.1)	$p>0.05$
C-peptide 120 min, ng/ml	4.9 (3.9; 5.5)	4.4 (3.8; 5)	$p>0.05$
Insulin 0 min, U/l	9.4 (5.3; 12.4)	6.9 (5.3; 8.1)	$p>0.05$
Insulin 60 min, U/l	33 (22.7; 38.9)	25.1 (18.2; 35)	$p>0.05$
Insulin 120 min, U/l	21.4 (19.7; 30.8)	22.4 (16.4; 38.2)	$p>0.05$

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Transcription activity testing can aid bioinformatics in ascribing pathogenicity to HNF1A variants identified by genomic sequencing in population-based cohorts

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Background and aims: In a population sample of randomly selected individuals from 3 different cohorts, as much as 2% carry rare non-synonymous variants in 1 of 7 maturity-onset diabetes of the young (MODY) genes. Interestingly, the majority of these individuals remain euglycemic through middle age. To evaluate their risk for T2D later in life, detailed analyses are required to determine the true pathogenic nature of these MODY gene variants, in addition to family pedigrees/phenotype analyses combined with bioinformatics *in silico* tools. Our aim is to test whether functional characterization of 25 rare variants (MAF<1%) in the hepatocyte nuclear factor 1 alpha (HNF1A) gene identified in randomly selected population cohorts, can aid bioinformatics to determine whether the variant is pathogenic or not.

Materials and methods: We classified the HNF1A variants into 5 groups: 1, not pathogenic; 2, likely not pathogenic; 3, uncertain; 4, likely pathogenic; 5, definitely pathogenic, by the mutation analysis tools PolyPhen-2, AlignGVGD og SIFT. Transactivation (TA) of HNF-1A (wild-type and mutants) was measured by luciferase assays of transiently transfected HeLa cells. Protein expression levels were confirmed by immunoblotting. Investigations of their DNA binding ability (by electrophoretic mobility shift assay) and subcellular localization are pending.

Results: Of 25 HNF1A variants, none were in class 5, 2 were in class 4, 14 in class 3 and 9 in class 2. Three variants had transcriptional activity of 0-40%, and all were associated with diabetes (two were in class 4). Four variants had transcriptional activity of 70-100%, all were class 2 or 3. Three of these were not associated with diabetes, while one was found both in diabetic and non-diabetic individuals. Eighteen variants had transcriptional activity of 40-70%, 6 were associated with diabetes, the others not.

Conclusion: Functional characterization by measurement of transcriptional activity for 25 HNF1A rare variants can supplement bioinformatics in ascribing pathogenicity, and seems to be most helpful for variants in class 3.

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Endocytosis of secreted carboxyl-ester lipase protein in CEL-MODY, a syndrome of diabetes and pancreatic exocrine dysfunction

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Background and aims: The carboxyl-ester lipase (CEL) gene, encoding the pancreatic enzyme CEL, is highly expressed in the pancreatic acinar cells. A dominant-acting frame-shift mutation (c.1686delT) in the last exon of CEL causes CEL-MODY (MODY8), a disease characterized by diabetes and pancreatic exocrine dysfunction. The resulting mutant CEL protein (CEL-MUT) has an altered, repetitive C-terminus leading to a higher tendency to aggregate and to undergo endocytosis as compared with the wild-type CEL protein (CEL-WT). We aimed to investigate the cellular reuptake of the mutant CEL protein and characterize its effect on cellular functions in acinar and β -cell models.

Materials and methods: HEK 293 cells stably expressing CEL-MUT and CEL-WT proteins were used to produce conditioned medium for use in endocytosis studies in acinar cells (rat AR42J and mouse 266-6) and β -cell models (rat INS-1E and mouse MIN6). We employed immunofluorescence confocal microscopy and assays of cell viability and glucose-stimulated insulin secretion.

Results: In stably transfected HEK293 cells, CEL-MUT was distributed in a cytoplasmic punctate pattern, whereas the CEL-WT predominantly localized typically for secreted proteins in the Golgi compartments and the endoplasmic reticulum. When untransfected acinar cells and β -cells were grown in conditioned medium from CEL-expressing cells, an accumulation of the CEL-MUT protein was observed in punctate structures. Long-term incubation of acinar and β -cells in conditioned medium showed a tendency, however not significant, of lower viability in the presence of CEL-MUT compared with CEL-WT. Glucose-stimulated insulin secretion in MIN6 cells was not reduced in the presence of the mutant protein.

Conclusion: Our results suggest that CEL-MUT can be endocytosed both by acinar and β -cells. The endocytic process might represent a mechanism of protecting cells against the exposure to CEL aggregates by cell-mediated uptake and degradation of the potentially toxic protein. Furthermore, endocytosis could be directly relevant for how the CEL-MUT causes disease affecting both the exocrine and endocrine pancreas.

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Wolfram syndrome in the Japanese population: molecular analysis of the *WFS1* gene and characterisation of clinical features

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Background and aims: Wolfram syndrome (WFS) is a recessive neurologic and endocrinologic degenerative disorder, and is also known as DIDMOAD (Diabetes Insipidus, early-onset Diabetes Mellitus, progressive Optic Atrophy and Deafness) syndrome. Most affected individuals carry recessive mutations in the Wolfram syndrome 1 gene (*WFS1*). However, the phenotypic pleiomorphism, rarity and molecular complexity of this disease complicate our efforts to understand WFS. To address this limitation, we aimed to describe complications and to elucidate the contributions of the *WFS1* mutations to clinical manifestations in patients with WFS.

Materials and methods: The minimal ascertainment criterion for diagnosing WFS was having both early onset diabetes mellitus (DM) and bilateral optic atrophy (OA). Genetic analysis for the *WFS1* was performed by direct sequencing.

Results: Sixty-seven patients were identified nationally for a prevalence of one per 710,000, with 33 patients (49%) having all 4 components of DIDMOAD. In 40 subjects who agreed to participate in this investigation from 30 unrelated families, the earliest manifestation was DM at a median age of 8.7 years, followed by OA at a median age of 15.8 years. However, either OA or DI was the first diagnosed feature in 6 subjects. In 10, features other than DM predated OA. Twenty-seven patients (67.5%) had a broad spectrum of recessive mutations in the *WFS1*. Two patients had mutations in only one allele. Eleven patients (27.5%) had intact *WFS1* alleles. Ages at onset of both DM and OA in patients with recessive *WFS1* mutations were indistinguishable from those in patients without *WFS1* mutations. In the patients with predicted complete loss-of-function mutations, ages at the onsets of both DM and OA were significantly earlier than those in patients with predicted partial-loss-of function mutations.

Conclusion: This study emphasizes the clinical and genetic heterogeneity in patients with WFS. Genotype-phenotype correlations may exist in patients with the *WFS1* mutations, as demonstrated by the disease onset.

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Czech prediction programme for type 1 diabetes: additional source of MODY patients?

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Background and aims: Since 2001, we prospectively follow up first-degree relatives of patients with Type 1 Diabetes (T1D) in the Czech Prediction Programme. Monogenic forms of diabetes, in particular Maturity-Onset Diabetes of the Young (MODY), are estimated to be responsible for 1-2% of all patients with diabetes. However, the MODY occurrence could be largely underestimated because it bears several clinical features common to other types of diabetes. We aimed to search for families with MODY among participants of Prediction Programme for T1D having positive family history of diabetes.

Materials and methods: First-degree relatives of patients with T1D are investigated for HLA-DQ genotype and annually screened for pancreatic autoantibodies (IA-2Ab, GADAb, IAA). Among 557 families included in the Prediction Programme for T1D, a positive family history of diabetes (at least 2 affected family members) was reported in 53 families (9.5%). In these families, one proband with diabetes was chosen for mutation screening of most prevalent MODY genes (*GCK*, *HNFI1A*, *HNFI4A* and *INS*) by direct Sanger sequencing.

Results: Of the 53 families with positive family history of diabetes in the Prediction Programme for T1D, 24 (45%) were genetically diagnosed with MODY. This makes up 4% of all the families from the Prediction Programme

for T1D. The most frequent mutations in our study were detected in the *GCK* (58%), followed by *HNF1A* (38%) and *INS* (4%) genes. The family-specific mutations were subsequently observed in 25/27 (93%) participants (first-degree relatives of patients with T1D) studied in the Prediction Programme for T1D: they were asymptomatic with exception of two children with *HNF1A*-MODY who presented osmotic symptoms (polyuria, polydipsia) during the follow up requiring insulin treatment which could be switched to administration of suphonylurea derivatives. Comparing MODY to non-MODY families with positive family history of diabetes, mothers of subjects in the Prediction Programme had more often diabetes than fathers and siblings ($p<0.0001$), the pancreatic islet autoantibodies were absent in all subjects with MODY ($p=0.009$) and the risk DQA1 and DQB1 alleles were less frequently seen in children from MODY families followed in the Prediction Programme ($p=0.02$).

Conclusion: This systematic search for MODY patients in the Prediction Programme for T1D has shown that monogenic diabetes accounts for a substantial number of cases with diabetes which could be higher than estimated previously. MODY should be considered in all families with history of diabetes because they may benefit from the optimization of the therapeutic options, prediction of disease prognosis and identification of diabetes risk in family members.

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MODY is uncommonly diagnosed in the South Asian ethnic group as a result of difficulty in differentiating young-onset type 2 diabetes from MODY

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Background and aims: Maturity onset diabetes of the young (MODY) has been extensively described in white-European (WE) populations, however comparatively few cases have been found in other ethnic groups. It is unclear if this reflects a genuinely lower prevalence of MODY in other ethnic groups or a disparity in testing-rates. We examined South Asian (SA) referrals for MODY testing in the UK.

Materials and methods: A retrospective cross-sectional analysis of UK patients referred for MODY genetic testing was undertaken (*HNF1A*, *HNF4A* and *GCK*). Ethnic origin, results of genetic analyses and clinical and biochemical characteristics were examined on all referrals, and sub-analysed by mutation result.

Results: 26 SA and 836 WE patients had mutations in one of the common MODY genes (*HNF1A*, *HNF4A* and *GCK*). This low SA prevalence of genetically confirmed MODY did not reflect a low referral rate, as 207 of 3491 (5.9%) referrals were SA, matching UK census data (5.3% population). However the mutation detection rate in the SA group was half that seen in the 3284 WE referrals (12.6% vs 25.5%, $p<0.001$). To analyse why the mutation detection rate was lower, we compared the clinical phenotype of SA and WE patients who a) had a genetic diagnosis of MODY and b) in whom a MODY mutation was not detected. a) Mutation phenotypes were similar between ethnic groups; SA *HNF1A* patients had similar age at diagnosis (18.0 vs 15 years, $p=0.072$), proportions of parental diabetes (100% vs 87.8%, $p=0.244$) and non-insulin requirement (80 vs 66.5%, $p=0.30$) to WE *HNF1A* patients. SA *GCK* patients were younger at diagnosis (12 vs. 19.5 years, $p=0.008$), with similar proportions of parental diabetes (85.6 vs. 74.5%, $p=0.303$) and non-insulin requirement (100 vs. 90.6%, $p=0.344$) to WE patients. b) Phenotypes in those tested for MODY in whom no mutation was found differed markedly between ethnic groups; SA subjects were younger at diagnosis (20 vs 25 years, $p<0.001$), with similar BMI (25.0 vs 25.2, $p=0.198$), were more likely to have an affected parent (77.3% vs 57.5%, $p<0.001$) and to be non-insulin treated (62% vs 52.1%, $p=0.007$) when compared to WE patients. The strongest predictors of MODY mutations in SA people were younger age at diagnosis (14.5 vs. 20 years, $p=0.001$) and lower BMI (20.0 vs 25.0 kg/m², $p<0.001$) when compared to SA people without mutations.

Conclusion: The reduced prevalence of molecular genetically diagnosed MODY in SA people results from a lower mutation detection rate, rather than a reduced referral rate. The low detection rate in the SA group reflects the higher prevalence of early-onset, non-insulin requiring, familial diabetes that does not result from MODY mutations. Differentiating MODY from young-

onset type 2 diabetes in people of SA origin is challenging, and further work is required to develop ethnic specific targeted referral criteria.

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Genetic testing for monogenic diabetes using targeted next-generation sequencing in the MODY registry cohort of Poland

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Background and aims: Specific molecular diagnosis of monogenic diabetes mellitus is important for individualized patient care. When results of standard Sanger sequencing in selected genes are negative, Next Generation Sequencing-based exome - sequencing (NGS), might provide additional diagnostic potential. NGS enables the simultaneous analysis of multiple genes in a single test. Our aim was to assess the feasibility of using NGS for a detection of mutations in a set of earlier described monogenic diabetes genes in a group of patients from the Polish Registry of MODY.

Materials and methods: We selected 29 genes (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *NEUROD1*, *INS*, *CEL*, *PDX1*, *PAX4*, *BLK*, *KLF11*, *KCNJ11*, *ABCC8*, *PTFA1A*, *NEUROG3*, *RFX6*, *EIF2AK3*, *FOXP3*, *GLIS3*, *SLC19A2*, *SLC2A2*, *IER31P*, *ZFP57*, *WFS1*, *GATA6*, *GATA4*, *LMNA*, *PPARG*) in which mutations have been reported to cause MODY, neonatal diabetes, maternally inherited diabetes and deafness (MIDD) or familial partial lipodystrophy (FPLD). The Illumina MiSeq platform was used for sequencing. An exon-capture assay was designed to include coding regions and splice sites, and it was based on the assay designed by the Exeter group. A total of 41 patients were selected from our cohort of suspected MODY. Sequence data were analysed for the presence of base substitutions, small insertions or deletions (indels) and exonic deletions or duplications.

Results: Sequencing results were generated for all 41 patients. In two positive controls, we detected previously identified *HNF1A* and *GCK* mutation. We detected 2 known *HNF1A* gene mutation and 1 known *GCK* gene mutation (missed in Sanger sequencing) in patients previously examined with negative results towards *HNF1A* and *GCK* MODY, respectively. In the other 24 patients previously negatively tested for *HNF1A* or *GCK* MODY we found high or moderate impact mutations in *GCK* (4), *HNF4A* (2), *ABCC8* (2), *HNF1A* (1), *NEUROD1* (1), *ZFP57* (1), *WFS1* (1), and *GATA6* (1) gene. We also included 12 patients with no prior genetic testing. In this subgroup we identified known mutations in *HNF1A* (3), *GCK* (3), *ZFP57* (1) and *LMNA* (1) gene. Currently, we are in the process of verification the sequence differences and their segregation in families by the Sanger sequencing.

Conclusion: Our pilot experiments using NGS for monogenic diabetes screening in the MODY cohort confirmed that its use is feasible in routine genetic testing. The screening should include patients in whom MODY is suspected and in whom earlier Sanger-based screening for MODY generated negative results.

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DNA testing of MIDD in patients with clinical suspicion on MODY or MIDD in Slovakia

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Background and aims: Diabetes and deafness - the leading clinical features of MIDD (Maternally Inherited Diabetes and Deafness) arise from mutations in mtDNA, most often m.3243A>G. This mutation leads to different clinical symptoms according to heteroplasmy levels in different tissues. Usually, the first presentation of MIDD is a sensorineural hearing loss emerging in adolescence; the diabetes mostly develops between 20th-40th year of life. More severe clinical presentation of the mutation is the MELAS syndrome (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like epi-

sodes). The aim of our study was to search for m.3243A>G mutation among patients sent for MIDD or MODY testing, who lack mutations in the most common MODY genes (i.e. GCK, HNF1A or HNF4A).

Materials and methods: Unrelated probands from 257 families fulfilling at least one of the following criteria, i.e. matrilinear inheritance, diabetes plus hearing impairment, diabetes development after 20th year of life, or progressive hearing loss, were tested for m.3243A>G mutation by the RFLP and/or Real-Time PCR. The heteroplasmy was evaluated for peripheral blood and/or buccal mucosa. DNA testing was also extended to the family members of probands carrying the mutation.

Results: The m.3243A>G mutation was found in 18 patients from 8 families (3%). Probands' phenotypes varied from diabetes as the sole symptom to a complex picture of the MELAS syndrome (in one proband). Diabetes or impaired glucose tolerance developed all of the probands (diabetes onset ranged from 21 to 52 years), but only 3 of 10 relatives with the mutation. Five probands (62.5%), and 4 (40%) of the relatives with the mutation had hearing impairment. The heteroplasmy was higher in buccal swab samples compared to the peripheral blood ($27.9 \pm 18.6\%$ versus $12.7 \pm 17.3\%$). In one case, the heteroplasmy was detected in the buccal DNA only, while the blood DNA samples were repeatedly negative.

Conclusion: Among 257 probands with the clinical suspicion on MIDD or MODY, 8 (3%) had the m.3243A>G mutation of the mitochondrial DNA. At the time of testing, only 5 (62.5%) of the probands were diagnosed with typical combination of symptoms, i.e. diabetes and hearing loss. Therefore, DNA testing for MIDD seems to be reasonable also in diabetes patients without hearing impairment, particularly using DNA from the buccal mucosa.

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Using targeted sequencing to investigate the prevalence of monogenic diabetes in the Norwegian childhood diabetes registry

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Background and aims: Type 1 diabetes is a heterogeneous group of diabetes. Since about 10–15 % lack detectable levels of diabetes associated autoantibodies at diagnosis, we hypothesized that many of these may have a monogenic form of diabetes. We therefore set out to systematically screen all auto-antibody negative patients in the Norwegian Childhood Diabetes Registry for 13 genes known to cause monogenic diabetes. The aim of this study was to estimate the prevalence of common forms of monogenic diabetes in childhood diabetes and assess the usefulness of large scale gene sequencing in routine clinical care of young onset diabetes.

Materials and methods: The Norwegian Childhood Diabetes Registry includes 95% of all children with newly diagnosed diabetes from 2002. By 22.01.2014, the registry included 3578 children with clinical data regarding onset of diabetes, family history and treatment, as well as serum and DNA samples. In this study, we selected 478 cases that were negative for GAD and IA-2 autoantibodies, as well as 478 GAD and IA-2 antibody-positive individuals as controls. The samples were screened by TruSeq Custom Amplicon targeted sequencing method, focusing on 13 monogenic diabetes genes (*HNF1A*, *HNF4A*, *GCK*, *NEUROD1*, *ABCC8*, *KCNJ11*, *HNF1B*, *INS*, *PDX1*, *KLF11*, *PAX4*, *BLK*, *CEL*). The amplicons targeted the protein coding regions with flanking intronic sequences, UTRs and some selected regions; *HNF4A* (c.-192), *INS* intron (c.188-31) and *GCK* (c.-71). To test the sensitivity of the assay, we tested 22 controls with known mutations in the selected target genes. We identified all these mutations. Sequencing was performed on a Miseq. All newly identified mutations were confirmed by Sanger sequencing. We used standard bioinformatics tools to assess the pathogenicity of the variants identified.

Results: As a first test, we screened 74 antibody negative cases that were C-peptide positive (C-peptide > 200 pmol/l at diagnosis) to increase the likelihood of finding possible MODY. In the screened material we found 39 exonic and splice variants whereof twelve of the patients (16%) had a likely pathogenic mutation in *HNF1A* or *GCK*. There were no false positive variants reported by the assay. Mean exonic coverage was estimated to 505X and

94% of target exons were covered at 20X. We are currently in the process of analyzing the remaining samples.

Conclusion: In this preliminary study where we use targeted sequencing to investigate the prevalence of monogenic diabetes in the Norwegian Childhood Diabetes Registry we observe that 16% of the GAD- and IA-2-antibody negative patients harbor a likely pathogenic mutation. This panel-sequencing assay provides a specific and sensitive method for simultaneous analysis of 13 genes associated with monogenic diabetes.

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Clinical features of MODY2 in children and adolescents in Russian Federation

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Background and aims: Maturity-onset diabetes of the young, type 2 (MODY2) is a monogenic disease caused by mutations in the glucokinase gene (GCK). Aim: To determine the prevalence of GCK gene mutations and to delineate the clinical phenotype of identified GCK mutation carriers in monogenic diabetes patients in Russia.

Materials and methods: We included 96 patients with suspected MODY. All patients were screened for HNF1A and GCK mutations by direct sequencing. **Results:** Mutations in the GCK were found in 46 patients (48 %), and HNF1A mutation - in 18 patients (19%), 32 patients (33%) were negative for MODY2 and MODY3. The age at diagnosis was 10.4 yrs (7;14). 11% of patients were investigated because of clinical manifestations, 66% were diagnosed occasionally, 23% were screened because of family history. Duration of the disease was 1.3 (0.6;2.3). BMI was in normal range in 95.6% of the patients (17.0 (15.8;18.5)). BMI was increased to 28.8 (SDS BMI +3.2) in only one 8-year-old girl. HbA1c at the time of diagnosis was 6.6% (6.4;6.8). autoantibodies (ICA, GAD, IAA) were negative in all patients. GTT: Glu (mmol/L) 0' -6.6 (5.9; 7), 1-h -11.15 (9.2; 12.78), 2-h -9.15 (8.475; 10.35). Seven patients (15%) aged 11.8–13.2 yrs with normal BMI were found to be insulin-resistant (Insulin at baseline was 14.2 U/l (11.0;15.2), HOMA > 3.5). Three patients requested insulin therapy (6.5%), and three other patients received sulfonylureas. 87% (40 patients) were able to maintain normal blood glucose levels with a diet and physical exercise only.

Conclusion: MODY3 is seemed to be less prevalent than MODY2 in Russian population. Most of the patients have asymptomatic disease and have been revealed occasionally in our study by impaired fasting glucose and slightly elevated HbA1c. Treatment for the majority of MODY2 children can be restricted by diet and physical exercise.

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The length of the deletion in the region 17q contributes to the individual variability of the phenotype of patients with renal cysts and diabetes syndrome (RCAD, HNF1B-MODY)

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Background and aims: The renal cysts and diabetes (RCAD) syndrome caused by defects in a gene for hepatocyte nuclear factor 1 beta (HNF1B) is characterized by broad spectrum of clinical features. While heterozygous point mutations detectable by Sanger sequencing are relatively rare, we focused on gross deletions of the HNF1B gene that are determined by MLPA (Multiplex Ligation Probe-dependent Amplification). Rather importantly, the deletions most often extend beyond the single HNF1B gene, thus more deleted genes may participate in the clinical picture. Objective and hypotheses. We compared the clinical phenotype of patients carrying the gross deletions whose extent was precisely determined by array comparative genomic hybridisation (aCGH), and point mutations in the HNF1B gene.

Materials and methods: In thirteen patients (6 males, median age 15.5 years) carrying the HNF1B gene deletion was performed aCGH on CytoChip Oligo 8x60K. The clinical data were compared with 5 patients (1 male, median age 15.5 years) having point mutations in HNF1B.

Results: The average length of heterozygous deletion was 1.69 Mb. The longest deletion reached 2.5 Mb affecting 47 genes and the shortest deletion found in three patients was 1.4 Mb long and deleted 16 genes. All patients lost also LHX1 gene encoding transcription factor important for the development of the renal and urogenital system. Compared to other deletion carriers, patients having longest deletions (2.5 and 2.1 Mb) manifested renal dysfunction at older age (10 and 30 years) with milder changes of the kidney structure (isolated cysts and functional changes only) and both presented diabetes as a first clinical feature of RCAD. Patients with shorter deletion manifested renal changes (polycystosis) prenatally and are mostly without diabetes so far. Comparing deletion and point mutation carriers, prenatal ultrasound kidney changes were found in 10/13 and 4/5 patients, respectively. Diabetes manifested at the median age of 17 years in 5/13 and 2/5 patients. Hypomagnesaemia was present in 11/13 and 2/5 patients.

Conclusion: Regardless to the length of the deletion in the region 17q, the dominating clinical phenotype of the patients is similar compared to the patients having point mutation in the HNF1B gene. However, the length of the deletion can contribute to the individual variability in the age of manifestation and other variability of the phenotype.

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PS 013 Islet gene expression in type 2 diabetes

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Beta cell function, more than beta cell area, affects plasma glucose levels and influences the progression of type 2 diabetes in humans

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Background and aims: Type 2 diabetes (T2D) is characterized by reduced beta cell functional mass. However, the direct relationships between beta cell amount or function with *in vivo* blood glucose levels and disease progression are unclear.

Materials and methods: Pancreases were obtained from 388 non-diabetic (ND) donors [age: 60±17 years, BMI: 25.2±3.8 kg/m², mean glycemia (MG) in intensive care unit: 154±45 mg/dl] and 68 T2D subjects [(age: 71±8 years, BMI: 26.9±4.0 kg/m², MG: 218±72 mg/dl, diabetes duration (DD): 9±7 years)]. Pancreatic insulin area (PIA) was assessed by immunocytochemistry; islets (HI) were isolated and *ex-vivo* insulin release (IR) was evaluated in response to 3.3 and 16.7 mM glucose (G).

Results: PIA was lower in T2D than ND subjects (0.51±0.20 vs 0.84±0.42%, $p<0.001$). IR (μU/islet/min) in response to 3.3 mM G (0.031±0.010 vs 0.036±0.015, $p=0.004$) and 16.7 mM G (0.050±0.030 vs 0.104±0.077, $p<0.001$) was also reduced in T2D islets, which was confirmed by calculation of stimulation index (SI, 1.6±0.7 vs 2.8±1.7, $p<0.001$). MG was not correlated with PIA ($p=0.30$), but it was inversely correlated with IR at 16.7 mM G ($p=0.002$) and SI ($p=0.001$). Anti-diabetic therapy consisted of diet alone (D) in 3 subjects, metformin (M) in 15, sulfonylurea alone or in combination with metformin in 25, insulin associated with oral agents in 6, insulin alone (I) in 9. Unsurprisingly, progression of therapeutic treatment was correlated with DD ($p<0.001$). There was no significant correlation between PIA and DD or pharmacological therapy. However, lower glucose stimulated IR was associated with progressively more intense diabetes therapy ($p<0.001$). Correlations were validated by multivariate analysis, and comparisons between therapeutic groups confirmed differences in terms of DD (from 6±3 yrs in D+M to 17±10 yrs in I), IR in response to 16.7 mM G (from 0.069±0.043 to 0.29±0.01 μU/islet/min) and SI (from 2.1±0.7 to 1.1±0.1) ($p<0.01$).

Conclusion: These results suggest that beta cell function, more than beta cell amount, affects plasma glucose levels and progression of T2D in humans; if so, improving beta cell function is a primary goal in T2D therapy.

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Uncovering conserved beta cell transcriptome of type 2 diabetes by meta-analysis of microarray data

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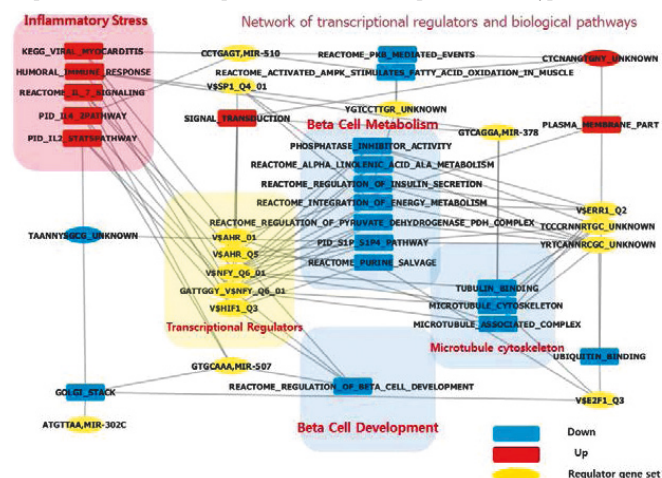
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Background and aims: Pancreatic beta cell dysfunction is an early manifestation during progression to diabetes. Transcriptional profiling studies of beta cells from subjects with diabetes have revealed various genes or pathways of beta cell dysfunction. We aimed to uncover conserved beta cell transcriptional signature in diabetes by meta-analyzing microarray datasets and find regulators.

Materials and methods: Microarray data of three independent transcriptional profiling studies of beta cells from diabetes patients (GEO IDs: GSE20966, GSE25724, and GSE38642) were obtained from the public microarray database, Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/geo/>). Gene Set Enrichment Analysis (GSEA) was performed for 10,294 curated mSigDB gene sets (<http://www.broadinstitute.org/gsea/msigdb>) in each microarray dataset and Enrichment Scores (ES) were calculated. As a measure of consistently altered expression, meta-Enrichment Score (Meta-ES) is defined as the sum of Enrichment Scores in three datasets. Gene sets with significantly high absolute meta-ES values ($P < 0.01$) are extracted. Next, we constructed the network of the significantly and consistently altered gene sets and regulators at the hub of the gene set network were assumed to be the key regulators of beta cell transcriptome of diabetes.

Results: 461 out of 10,294 mSigDB gene sets were significantly and consistently altered across datasets (P value of meta-ES <0.01). In gene set network, cytokine metabolism and immunologic response pathways were up-regulated, while beta cell differentiation, energy metabolism, and microtubule associated gene sets were down-regulated in all three datasets. Analysis of the network topology of transcription factor target gene sets and biological pathway/gene ontology gene sets suggests that HIF1A, aryl hydrocarbon receptor, and Nuclear Factor Y may be core regulators that account for the repressed beta cell development and metabolism of diabetes patients exposed to chronic metabolic and inflammatory stress (see attached figure).

Conclusion: Integrative meta-analysis of heterogeneous transcriptional profiling studies of beta cells from diabetes patients proposes that HIF1A, aryl hydrocarbon receptor, and Nuclear Factor Y may be candidate regulators of impaired beta cell development and function in patients with type 2 diabetes.



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Serotonin receptors HTR1D and HTR2A are differentially expressed in human type 2 diabetes islet donors and regulate insulin secretion in vitro in human islets and INS (832/13) cells

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Background and aims: Regulation of overall glucose homeostasis is achieved by an intricate interplay between metabolic and nervous input to the pancreatic alpha and beta cells. Failure of adequate signals to the beta cells may result in overt type 2 diabetes (T2D). Intra islet paracrine and autocrine circuits of the monoamine 5-hydroxy tryptamine (5-HT) is stored within the islets and can potentially regulate glucose stimulated insulin release (GSIS). Discrepancies in results of the effects of 5-HT on insulin release in vitro and in vivo and lack of studies in human islets demand further investigation of the islet 5-HT systems. Moreover, diabetogenic effects of atypical antipsychotics and antidepressants that specifically targets these systems urges increased understanding of effects directly on the islets. Therefore, in this study we aimed to perform a complete transcriptional mapping of 5-HT receptors, investigate differentially expressed receptors in non diabetic and T2D human islet donors, and to study the role for these receptors in insulin secretion.

Materials and methods: Islet donors were obtained from the Nordic Network of Clinical transplantation in Uppsala, Sweden. RNA sequencing data of 131 islet donors (114 control donors and 17 T2D donors) were used. Validation of RNA sequencing data for receptors was performed by quantitative (Q) PCR (Applied Biosystems). One hour batch secretion experiments were performed using secretion assay buffer (2.8 mmol/l glucose, 114 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 1.16 mmol/l MgSO₄, 25.5 mmol/l NaHCO₃, 20 mmol/l Hepes, 2.5 mmol/l CaCl₂, and 0.2% BSA), with or without addition of agonists and antagonists. Localization for receptors and amine was performed by immunohistochemistry in sections of human pancreas.

Results: We detected the expression of fifteen 5-HT receptors in human islets, as well as all enzymes involved in the biosynthesis of 5-HT (TPH1, TPH2 and DDC). Searching for differential receptor expression between non-diabetic and T2D islet donors, expression for HTR1D and HTR2A was found to be increased in T2D islets (p=0.0043 and p=0.0041). Batch incubations with human islets revealed that agonist for 5-HT1d (PNU142633) decrease GSIS while an agonist for 5-HT2a (TCB-2) potentiated GSIS. Conversely, an antagonist for 5-HT2a (EMV) decreased GSIS and an antagonist for 5-HT1d (LY3107609) increased GSIS. Immunohistochemistry localized 5-HT1d and 5-HT2a receptors and 5-HT to both human beta and alpha cells.

Conclusion: Our findings support a role for 5-HT in control of human islet hormone secretion, perhaps in a paracrine and autocrine fashion as we show that 5-HT is present in both human alpha and beta cells. Moreover, expression of HTR1D and HTR2A is changed in T2D islets, suggesting a role for these receptor in the pathophysiology of type 2 diabetes.

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Effects of steroid treatment on beta cell mass in Japanese individuals with or without diabetes

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Background and aims: Steroid is widely used anti-inflammatory agent. Steroid treatment promotes hepatic and peripheral insulin resistance and, in rodent studies, increases beta cell mass (BCM). Recently we have reported that there was no increase in BCM in Japanese obese individuals compared with lean subjects. Therefore in this study, we aimed to clarify the effects of steroid treatment on BCM in Japanese individuals with or without diabetes.

Materials and methods: We obtained the human pancreas at autopsy from 26 non-diabetic individuals (NGT; control) and 26 steroid-treated non-diabetic individuals (SNGT) (age 63 ± 8 (mean ± S.D.) vs. 60 ± 11 years, HbA1c 5.5 ± 0.6 vs. 5.3 ± 0.6%, respectively). We also obtained the pancreas from 23 steroid-treated individuals with diabetes (SDM) and 25 individuals with type 2 diabetes (DM2) (age 65 ± 8 vs. 66 ± 8 years, HbA1c 7.1 ± 1.0 vs. 7.5 ± 1.2%). To be included, cases were required to have 1) had a full autopsy within 24 h of death, 2) pancreas tissue stored with adequate size and quality. Cases were excluded if pancreas tissue showed autolysis or any abnormal change such as pancreatitis. Pancreatic sections were stained for insulin or glucagon, and fractional beta (%BCA) or alpha cell area (%ACA) was measured.

Results: There was no significant difference in %BCA between SNGT and NGT (1.21 ± 0.59 vs. 1.66 ± 1.05%, P = 0.29). In SNGT group, there was no significant correlation between %BCA and duration of steroid treatment (r = 0.404, P = 0.06), or total steroid dose (r = 0.354, P = 0.15). %BCA in DM2 was significantly decreased compared with NGT (0.92 ± 0.63%, P = 0.004), however there was no significant difference in %BCA between SDM and DM2 (0.99 ± 0.56%, P = 0.54). There was no correlation between %BCA and duration of steroid treatment (r = 0.065, P = 0.77) or total steroid dose (r = 0.077, P = 0.75) in SDM. When the SDM group were divided into two groups according to the presence (SDM-DM2, n = 13) or absence (SDM-NGT, n = 10) of type 2 diabetes, %BCA in SDM-NGT was significantly greater than that in SDM-DM2 (1.34 ± 0.53% vs. 0.72 ± 0.43%, P = 0.01) and comparable to that in NGT (P = 0.55). Although there was a significant negative correlation between %BCA and HbA1c in NGT plus DM2 group (r = -0.451, P = 0.004), this correlation was not observed in steroid-treated subjects (SNGT plus SDM groups) (r = -0.266, P = 0.08).

Conclusion: There was no increase in BCM in steroid-treated Japanese individuals with or without diabetes, suggesting that steroid treatment does not affect BCM in Japanese. No significant decrease in BCM in patients with steroid-induced diabetes suggests that the glucose intolerance in these subjects is mainly due to the presence of insulin resistance rather than the deficit of beta cell. Since we were not able to observe a significant increase in BCM in the presence of obesity or steroid use in Japanese, these findings suggest that the increase in BCM in the face of insulin resistance is extremely limited in Japanese.

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Dephosphorylation and nuclear translocation of the CREB-regulated transcription co-activator 1 (CRTC1) elevates miR-212 and miR-132 expression in insulin secreting cellsH.A. Malm¹, C. Berggreen², M. Orho-Melander¹, L. Eliasson¹, O. Göransson², I.G. Mollet²;¹Dept Clinical Sciences in Malmö, Lund University Diabetes Centre, Malmö,²Dept of Experimental Medical Sciences, Lund University, Lund University Diabetes Center, Sweden.

Background and aims: We have previously demonstrated miR-212 and miR-132 to be upregulated in diabetic GK rat islets. Others have demonstrated cAMP-responsive element binding protein (CREB) to bind CRE-sites on the miR-212/132 promoter. Here we aim to investigate mechanisms underlying cAMP-mediated transcriptional regulation of the miR-212/132 cluster in insulin secreting cells.

Materials and methods: INS-1 832/13 cells were cultured for 0.5, 2, 6 and 24 h in various glucose concentrations and in the absence or presence of forskolin/IBMX or GLP-1 (2 h). In addition, cells were transfected with siRNA against all three isoforms of CRTC or salt inducible kinases (SIKs) or with mature miR-212 and miR-132. Relative expression of miRNA and mRNA was determined by qPCR; Insulin secretion at 2.8 and 16.7 mM glucose was analyzed using RIA; protein expression and phosphorylation was evaluated with western blot analysis (WB) and CRTC1 translocation with confocal microscopy.

Results: Expression of both miRNAs was increased after 2 h in forskolin/IBMX (3–4-fold, $p < 0.01$) and increased further at 6 h. CREB was phosphorylated and CRTC1 was dephosphorylated ($p < 0.001$) after 30 min incubation with forskolin/IBMX. In addition, SIK2 was phosphorylated on Ser358 after forskolin/IBMX treatment ($p < 0.001$). In the presence of forskolin/IBMX, expression of miR-212 and miR-132 ($p < 0.05$) and insulin release at 16.7 mM glucose ($p < 0.01$, 50%) were decreased in CRTC1 silenced cells. Expression of CRTC1 mRNA was reduced in cells overexpressing miR-212 and miR-132 ($p < 0.05$), suggesting a feedback control system. Confocal imaging revealed an accumulation of CRTC1 in INS-1 832/13 nuclei in forskolin/IBMX-incubated cells ($p < 0.05$). Finally, expression of miR-132 was elevated in cells incubated with 10 ($p < 0.05$) and 100 nM ($p < 0.01$) GLP-1, respectively and expression of miR-212 was elevated in cells incubated with 100 nM GLP-1 ($p < 0.05$).

Conclusion: We have demonstrated that the incretin GLP-1 is an activator of miR-212 and miR-132 expression. Furthermore, our data suggest that miR-212 and miR-132 are regulated by cAMP in INS-1 832/13 cells, and that this is in part mediated through CRTC1 presumably acting together with CREB. We are currently further addressing the potential role of SIK isoforms as upstream regulators of the CRTC1/miR-212/132 pathway.

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Pdx1 coordinately cooperates with USF transcription factors to regulate expression of Alx3 in pancreatic beta cells

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Background and aims: Alx3 is emerging as an important transcription factor for the control of glucose and metabolic homeostasis. Alx3 deficiency in mice results in age-dependent pancreatic islet cell apoptosis. As a consequence, beta-cell mass and insulin content is reduced, and islet size is decreased. These mice exhibit elevated fasting blood glucose levels, impaired glucose tolerance, and insulin resistance that becomes evident as they age. These findings, together with its involvement in the regulation of the insulin gene, indicate that Alx3 is important for pancreatic islet function. For this reason, we embarked in a series of studies aimed to investigate the transcriptional mechanisms that promote Alx3 expression in pancreatic islet cells. We have previously reported that the transcription factors USF1 and USF2 bind to different E-box elements in the promoter of Alx3 to regulate basal expression in mesenchymal and pancreatic cells. In the present study, we aimed to identify additional transcription factors involved in Alx3 expression in beta cells.

Materials and methods: Putative regulatory elements were identified by analysis of the promoter in silico. Binding of nuclear proteins from isolated mouse islets or from MIN6 cells was investigated by chromatin immunoprecipitation and electrophoretic mobility shift assays. To assess the role of spe-

cific transcription factors on the expression of Alx3 we used siRNA. Expression of Alx3 was assessed by quantitative RT-PCR. Luciferase reporter genes were used to test for promoter activity in transfected cells.

Results: The functional importance of USF1 and USF2 in the regulation of the endogenous Alx3 gene was confirmed in MIN6 cells by silencing their expression using siRNA. We found that silencing USF1 and USF2 concomitantly decreased Alx3 expression by approximately 50%. In silico analysis of the Alx3 promoter led to the identification of the conserved sequence TTAATGA, a potential binding site for the pancreatic transcription factor Pdx1. Mutation of this site resulted in decreased promoter activity in transfected MIN6 cells, and diminished Pdx1-dependent transactivation in Hela cells. ChIP assays using chromatin from isolated mouse islets, and EMSA using nuclear extracts from MIN6 cells confirmed binding of Pdx1 to the Alx3 promoter. In addition, silencing Pdx1 expression by siRNA resulted in decreased Alx3 expression in MIN6 cells. Interestingly, mutations of the USF transcription factor-binding E-boxes almost completely inhibited Pdx1-dependent transactivation of the Alx3 promoter. A similar effect was observed when Pdx1 was cotransfected with a dominant negative inhibitor of USF transcription factors. Finally, coexpression of USF1, USF2 and Pdx1 resulted in enhanced Alx3 promoter activity.

Conclusion: Expression of Alx3 in pancreatic islet cells is regulated by Pdx1. USF1 and USF2 transcription factors enhance Pdx1 transcriptional transactivation from binding sites located on at least three different E-boxes. These data indicate that Pdx1, USF1 and USF2 coordinately interact to maintain Alx3 gene expression in pancreatic islet beta cells.

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Islet insulin resistance promotes local apolipoprotein CIII production and beta cell failureK. Ávall¹, Y. Ali¹, L. Selander¹, S.K. Nilsson², R.M. Crooke³, M.J. Graham³, P.-O. Berggren¹, L. Juntti-Berggren¹;¹The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Stockholm, ²Department of Medical Biosciences, Umeå University, Sweden, ³ISIS Pharmaceuticals, Carlsbad, USA.

Background and aims: The progression of type-2 diabetes (T2D) occurs in phases. The initial phase is characterized by peripheral tissue insulin resistance with a β -cell compensatory response of increasing mass and insulin secretion. With prolonged hyperinsulinemia the β -cells start to fail, causing defects in insulin secretion and eventually increased β -cell apoptosis. Expression of apolipoprotein CIII (apoCIII) is increased under conditions of insulin resistance and recently it has been shown that hyperglycemia induces apoCIII transcription. The aim of the present study was to test the hypothesis that islet insulin resistance leads to local production of apoCIII and consequent β -cell failure in T2D.

Materials and methods: RNA was isolated and the expression of genes was measured by real-time quantitative PCR in islets from ob/ob and ob/lean mice at different ages. ApoCIII was lowered in vivo and in vitro by antisense treatment. Islets from ob/ob, C57Bl/6 and apoCIII^{-/-} mice were transplanted into the anterior chamber of the eye (ACE) of ob/ob or C57Bl/6 mice. Changes in [Ca²⁺]_i were measured in islets microdissected out from the ACE and the effect of five months high fat diet (HFD) was studied longitudinally by in vivo imaging.

Results: ApoCIII was produced locally in the islets and there was a three-fold increase in islets from 12-weeks old diabetic ob/ob mice compared to ob/lean mice. Lowering of apoCIII with antisense in vivo led to reduced body weight and improved glucose tolerance. Lowering apoCIII in vitro in isolated islets resulted in normalized Ca²⁺-handling and decreased caspase 3/7 activity, as a measure of apoptosis. ApoCIII^{-/-} islets transplanted into ob/ob mice with high systemic apoCIII levels showed normal Ca²⁺-responses, compared to transplanted ob/ob islets with increased endogenous apoCIII levels. Transplantation of islets from C57Bl/6 mice into the ACE of one eye of HFD fed mice showed an increased size compared to apoCIII^{-/-} islets transplanted into the other eye of the same mouse.

Conclusion: Insulin resistance in the pancreatic islet leads to local expression of apoCIII and compromised β -cell function and survival that can be restored/prevented by reducing the lipoprotein in vivo. Hence, under conditions of islet insulin resistance locally produced apoCIII is an important diabetogenic factor and may constitute a novel target for the treatment of T2D.

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Intracellular cholesterol transporters, islet dysfunction and insulin secretion in type 2 diabetes mellitus

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Background and aims: Elevated intracellular cholesterol levels prevent glucose stimulated insulin secretion from pancreatic beta cells, by impairing iCa^{2+} elevation and its signaling, and hindering PIP_2 hydrolysis, affecting actin dynamics and plasma membrane potential. Conversely, cholesterol depletion restores exocytosis of insulin granules in sterol-loaded cells. Mechanisms that improve the efficiency of intracellular cholesterol transport may therefore prove valuable therapeutically. Cholesterol-binding members of the steroidogenic acute regulatory protein domain (StarD) family of lipid trafficking proteins (STARTs) are known to modulate lipid homeostasis in a number of differing cell types, including macrophages, keratinocytes and hepatocytes; however, despite their potential therapeutic utility, little is known about the expression or function of StarD proteins, or their ligands, in beta-cells, or their relationship with insulin secretion. Our aims were to (i) investigate the expression of StarD cholesterol trafficking proteins, in BRIN-BD11 insulinoma cells, following manipulation of cellular cholesterol levels, and (ii) to explore the role of endosomal cholesterol transfer protein, StarD3, in cholesterol homeostasis and insulin secretion.

Materials and methods: Methodologies included radioisotopic labelling of cellular lipid pools, colorimetric assays for lipid mass, Q-PCR, immunoblotting and ELISA.

Results: Treatment with methyl beta-cyclodextrin (MCD; 1–10 mmol l⁻¹; 1 h) efficiently removed cholesterol from BRIN-BD11 cells, dramatically increasing endogenous cholesterol biosynthesis, while cholesterol lipid complex (CLC 1:200) induced significant increases in cholesterol mass ($p < 0.01$), without perturbing cholesterol biosynthesis. Secretion of insulin was modulated by cellular cholesterol content, exhibiting a biphasic response following MCD treatment, which was reflected in the expression of StarD3 protein, which increased at concentrations from 0.1 to 3 mM MCD, but declined markedly at 10 mM MCD. Sterol loading of cells also increased the expression of StarD3, compared with control cells, while StarD1 levels were too low in BRIN-BD11 cells for detection by immunoblotting. Levels of StarD4 protein showed a trend towards repression in cells treated with CLC, while StarD5 did not appear significantly altered under the conditions tested here.

Conclusion: The results suggest a possible relationship between StarD3 expression and insulin secretion, which are now being explored using the StarD3 ligand, lutein, and genetic manipulation of this protein, establishing the importance of targeting StarD proteins as tools to improve insulin secretion in beta cells.

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Low calorie sweeteners do not stimulate insulin secretion in human pancreatic beta cellsF. Bonnet¹, R. Scharfmann², E. Lepicard³, P. Czernichow⁴, G. Friedlander²;¹Dept. of Endocrinology-Diabetology, University Hospital, Rennes,²Faculté Necker, INSERM U845, Paris, ³Institute of European Expertise in Physiology (IEEP), Paris, ⁴Pépinère d'Entreprises Institut du Cerveau et de la Moelle, Endocells, Paris, France.

Background and aims: High obesity and diabetes rates highlight the need for individuals to balance calories consumed with calories burned through daily activities and exercise. Low calorie sweeteners can be a helpful tool to reduce energy intake and body weight in the context of a reduced-calorie diet, by offering an alternative to caloric sweeteners. However, it has been suggested that sweeteners can stimulate insulin secretion, increase sugar intestinal absorption and increase appetite, leading to hyperglycemia and weight gain. Our aims were firstly, to determine whether low calorie sweeteners modify insulin secretion, and secondly, to determine whether or not they stimulate glucose absorption by intestinal cells.

Materials and methods: Insulin secretion was investigated using a unique in vitro model of human pancreatic beta cells (EndoC betaH1). Low calorie sweeteners effects were investigated in the presence of basal (low, 2.8 mM) or high (11 mM) concentrations of glucose. Various low calorie sweeteners concentrations were tested including concentrations observed in commercially available soft drinks (1 mM). Glucose transport assays were performed using

0.1 mM or 1 mM of deoxyDglucose, a non-metabolized radiolabelled glucose analogue GLUT2-specific, in CACO2 cells or beta pancreatic cells.

Results: Aspartame, acesulfame K (both at 0.1, 1 mM and 10 mM) and blend of these two low calorie sweeteners (0.1/0.1 mM, 1/1 mM) did not induce insulin secretion in human pancreatic beta cells. This was observed not only in the presence of low but also of high concentrations of glucose, showing no potentiation of glucose effect upon human pancreatic beta cells. Moreover, both low calorie sweeteners did not increase human pancreatic beta cells proliferation or survival. A rise in insulin secretion was observed only in the presence of a very high dose of 50 mM of acesulfame K and the 20 mM aspartame/50 mM acesulfame K blend. Glucose uptake in both pancreatic and intestinal cells was not modified by aspartame, acesulfame K or blends of these two low calorie sweeteners up to 10 mM. At higher non physiological doses (10 mM or 20 mM), only aspartame reduced GLUT2-mediated glucose uptake in pancreatic beta cells ($p < 0.01$ for both doses of deoxy-D-glucose) and CACO2 cells (0.1 mM deoxy-D-glucose: $p = 0.002$; 1 mM deoxy-D-glucose $p = 0.0007$). Finally both low calorie sweeteners and the blend of low calorie sweeteners had no effect on GLUT2 expression.

Conclusion: At concentrations similar to those observed in commercially available soft drinks containing low-calorie sweeteners i.e. between 0.1 and 1 mM, aspartame and acesulfame K affect neither insulin secretion nor pancreatic beta cells survival and proliferation. In addition, both sweeteners alter neither glucose transport nor glucose transporters expression in both pancreatic and intestinal cells.

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PS 014 Beta cell proliferation and differentiation

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The pocket protein family involved in beta cell replication during pregnancy

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Background and aims: The pocket protein family consists of retinoblastoma protein (Rb), p107 and p130, which can repress E2F-mediated gene transcription, recruit chromatin remodeling enzymes, and undergo phosphorylation by cyclin-dependent kinases, comprising the central regulatory checkpoint that controls the mammalian G1/S-phase cell cycle transition. In spite of the sequence similarity within the DNA binding domain, they play distinct roles in the regulation of cell cycle progression. Islet beta cell replication is the main source for beta cell mass expansion during pregnancy. Activation of prolactin receptor signaling plays a key role in this process. Whether the pocket protein family is critical for beta cell replication during pregnancy is unclear. The present study is to investigate the change of Rb, p107 and p130 during pregnancy and its related mechanism in prolactin-mediated beta-cell replication.

Materials and methods: Pancreatic islets of C57BL/6 mice were isolated and purified by collagenase digestion at non-pregnant (NP), pregnant day 10.5 (P10.5), P14.5, P18.5 and after parturition day 4 (AP4), AP8. The gene and protein expression of Rb, p107, p130 and E2F1~5 were detected by real-time quantitative PCR and Western blotting methods. Rb and phosphor-Rb expression of islets were detected by immunofluorescence. INS-1 cells were transfected with siRNA mediated by liposome to knockdown Rb expression, and detected the change of cell cycle with prolactin stimulation by flow cytometry. INS-1 cells were cultured with inhibitors of AKT, Stat5, ERK, JNK and Pim respectively with prolactin stimulation to observe the change of Rb/E2F1 expression.

Results: During pregnancy, Rb, p107 and E2F1~3 gradually increased and returned back to NP level after parturition, with peak of Rb, phosphor-Rb and E2F1 at P14.5, p107 and E2F2 at AP4, E2F3 at P18.5 ($p<0.05$). p130, E2F4~5 reduced gradually during pregnancy, reached the bottom at p14.5~p18.5, then gradually returned back after parturition ($P<0.05$). Double staining of Rb and phosphor-Rb with insulin or glucagon respectively indicated that total Rb located in cytoplasm while phosphor-Rb located in nucleus. Rb and phosphor-Rb protein were expressed at NP, but strongly upregulated at P14.5 ($P<0.05$) and returned back to NP level after parturition. The mRNA and protein level of Rb, p107 and E2F1 in INS-1 cells increased significantly ($P<0.05$) while p130 decreased significantly ($P<0.05$) after prolactin stimulation. Rb knockdown in INS-1 cells by siRNA markedly increased prolactin-induced INS-1 cell proliferation with promoted G1/S-phase cell cycle transition ($P<0.01$). With the inhibitor of Stat5 and Pim respectively to block the signaling pathways, the protein expression of Rb and E2F1 were significantly decreased in prolactin-stimulated INS-1 cells ($p<0.05$). While inhibition of AKT, ERK and JNK signaling pathways did not influence Rb and E2F1 expression.

Conclusion: The expression of pocket protein family is dynamic changed during pregnancy. Rb is essential for G1/S-phase cell cycle transition in prolactin-induced INS-1 cell proliferation, which is the downstream of Stat5 and Pim pathway with prolactin receptor activation.

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Survivin mediated prolactin-induced INS-1 cell proliferation through both PI3K/AKT and STAT5 pathway

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Background and aims: Maternal pancreatic beta-cell mass increases dramatically during pregnancy both in rodents and humans to meet the increased physiological demands. This process is mainly due to beta-cell proliferation induced by activation of prolactin receptor signaling. Our previous data showed that Survivin was dynamic expressed in the pancreatic beta cells

during pregnancy. Islet beta-cell specific Survivin deletion mice displayed glucose intolerance during pregnancy with the defection in beta-cell proliferation and beta-cell mass expansion. However, the relationship between Survivin and the multiple putative signaling pathways downstream of the prolactin receptor is unknown. This study was designed to explore the function of Survivin in prolactin-mediated beta-cell proliferation.

Materials and methods: INS-1 cells were cultured with prolactin stimulation. Cell proliferation was detected by cell cycle analysis by flow cytometry. The gene and protein expression of IRS2/PI3K/AKT, Jak2/Stat5 and Foxm1/Survivin were detected by real time quantitative PCR and Western blotting methods. INS-1 cells were transfected with siRNA mediated by liposome to knockdown Survivin expression, and detected the change of cell cycle with prolactin stimulation. INS-1 cells were cultured with inhibitors of AKT and Stat5 respectively with prolactin stimulation to observe the change of Foxm1/Survivin expression.

Results: Cell cycle analysis showed that the proliferation of INS-1 cells increased significantly with prolactin stimulation ($P<0.01$). The mRNA expression of IRS2/PI3K/AKT, Jak2/Stat5 and Foxm1/Survivin were markedly upregulated ($P<0.05$). The protein expression of pAKT, pStat5 and Survivin were elevated at the same time ($P<0.05$). However, Survivin knockdown in INS-1 cells by siRNA markedly blocked prolactin-induced INS-1 cell proliferation with delayed S phase in cell cycle ($P<0.01$). With the inhibitor of AKT and Stat5 respectively to block the signaling pathways, the protein expression of Foxm1 and Survivin were significantly decreased in prolactin-stimulated INS-1 cells ($P<0.05$).

Conclusion: Survivin is crucial for prolactin-induced INS-1 cell proliferation. Foxm1/Survivin is the common downstream of both PI3K/AKT and STAT5 pathway with prolactin receptor activation.

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The non-canonical Wnt ligand, Wnt4, is highly expressed in pancreatic beta cells and its expression is negatively correlated with cell growth

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Background and aims: We have previously published data showing that in beta-cells most Wnt ligands are either not present or expressed at very low levels. The exception to this is Wnt4 which is expressed at levels 10 fold higher than any other Wnt ligand. Wnt4 has been suggested to have a negative effect on beta-cell biology, with the levels in islets increased in rodent models of insulin resistance. The aim of this study is to investigate the regulation of Wnt4 expression in beta-cells and the impact of Wnt4 on beta-cell growth.

Materials and methods: mRNA and protein expression levels were measured by qRT-PCR and western blotting.

Results: We investigated Wnt4 mRNA expression in mouse organs and found that islets expressed Wnt4 at 10 fold higher levels than kidney, liver, muscle or brain (Wnt4 mRNA expression relative to housekeeping genes; kidney: 0.01 ± 0.004 , liver: 0.01 ± 0.003 , brain: 0.02 ± 0.002 , muscle: 0.02 ± 0.009 and islets: 0.25 ± 0.018 , (mean \pm sem)), suggesting an important role for Wnt4 in islet cells. We used the rodent beta-cell line, INS-1 to investigate regulation of Wnt4 expression. In agreement with islet data, we found that INS-1 cells expressed high levels of Wnt4 mRNA and protein. To determine whether a diabetogenic environment altered Wnt4 expression, we treated INS-1 cells with high glucose (Wnt4 mRNA expression normalised to housekeeping genes and expressed relative to 5.5mM glucose; 5.5mM glucose: 1 ± 0.07 , 16.7mM glucose: 1.07 ± 0.13 , (mean \pm sem) $n=3$) or 0.25 and 0.5mM palmitate (Wnt4 mRNA expression normalised to housekeeping genes and expressed relative to control; control: 1.0 ± 0.06 , 0.25mM palmitate: 1.1 ± 0.1 , 0.5mM palmitate: 0.94 ± 0.06 , (mean \pm sem) $n=3$) for 24hrs. However none of these treatments altered Wnt4 mRNA expression. We did though find that as cell confluence increased (with an associated decrease in cell proliferation) levels of Wnt4 mRNA increased (Fig 1). In contrast the levels of the proposed Wnt4 receptor, Fzd6, remained unchanged (Fig 1). In addition levels of Wnt4 mRNA decreased upon 6hr treatment with the growth stimulating protein, HGF (Wnt4 mRNA expression normalised to housekeeping genes and expressed relative to control; control: 1 ± 0.08 , 10ng/ml HGF: 0.47 ± 0.07 , (mean \pm sem) $n=3$, $p<0.01$), suggesting a negative correlation between Wnt4 expression and beta-cell proliferation. In agreement with this we find that treatment of INS-1 cells with Wnt4 is able to inhibit cell growth stimulated by the canonical Wnt ligand, Wnt3a (% change in cell growth over 72hrs compared to control;

10ng/ml Wnt3a: $114.8 \pm 1.03\%$ ($p < 0.0001$), 10ng/ml Wnt4: $97.1 \pm 1.04\%$, 10ng/ml Wnt3a+10ng/ml Wnt4: $97.3 \pm 0.97\%$ (mean \pm sem), $n=4$).

Conclusion: Our data suggests that Wnt4 may act as a negative regulator of canonical Wnt signalling in beta-cells, leading to inhibition of beta-cell proliferation.

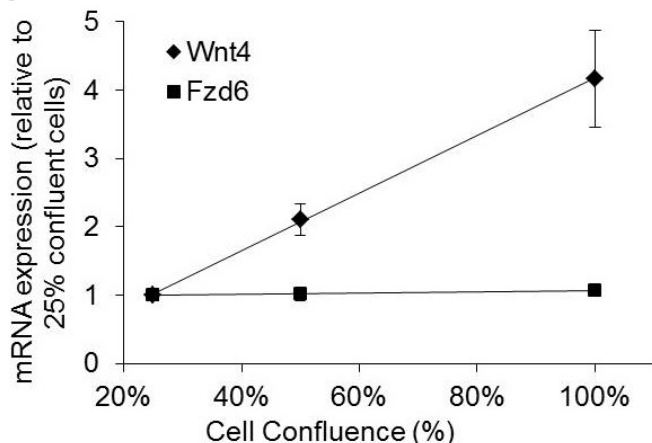


Figure 1. Regulation of Wnt4 gene expression: A: Cells were seeded at varying densities and mRNA extracted after 24hrs when at 25, 50 or 100% confluence. mRNA was converted to cDNA and used in qRT-PCR to measure Wnt4 and Fzd6 mRNA levels.

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Pancreatic cell fate decisions after beta cell specific insulin gene knockout in adult mice: a new model of acute beta cell dedifferentiation

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Background and aims: Pancreatic beta cell de-differentiation, including the loss of key regulatory gene networks, as well as the loss of insulin production and storage, has been associated with both type 1 and type 2 diabetes. Studies using mouse models of toxin-induced beta cell destruction have reported beta cell regeneration via beta cell proliferation or trans-differentiation of other cell types (such as alpha cells) leading to reversal of diabetes. Our aims were to define the potentially cell-autonomous role(s) insulin has in controlling beta cell fate, including differentiation/dedifferentiation, trans-differentiation, proliferation and apoptosis.

Materials and methods: We developed an inducible beta cell-specific insulin gene knockout model containing four engineered alleles, Pdx1-CreERT/-:mTmG/-:Ins1-/-:Ins2f/f. Cre-positive and Cre-negative littermates injected with vehicle or tamoxifen, respectively, were used as controls. Animals (6-8 weeks) were monitored for fasting blood glucose and plasma insulin levels for a 5 week or 6 day period. Pancreatic sections were used for immunohistochemical analysis for beta cell specific markers and markers for proliferation and apoptosis. Isolated islets were also harvested for RNA sequencing and qPCR.

Results: Tamoxifen-induced insulin gene deletion in pancreatic beta cells led to overt diabetes, marked by severe fasting hyperglycemia within 14 \pm 4 days in male mice (fasting blood glucose 29.6 ± 0.55 versus 8.7 ± 0.22 mM at 5 weeks, $p < 0.05$), with females having a delayed response (fasting blood glucose 17.6 ± 2.3 versus 8.13 ± 0.41 mM at 5 weeks, $p < 0.05$). Body weights remained stable throughout the study period in both sexes. The observed sex difference in diabetes incidence could be accounted for by differences in insulin sensitivity. As expected, plasma insulin levels were also significantly decreased to $38 \pm 2.7\%$ ($p < 0.05$) of pre-tamoxifen levels in diabetic male mice. A significant reduction in insulin positive beta cell number/area was evident when comparing diabetic mice to non-diabetic animals. Prior to and after reaching hyperglycemia, immunohistochemical analysis of pancreatic sections did not reveal obvious signs of widespread trans-differentiation. Preliminary data also showed a 1.83 fold increase in beta cell proliferation in Cre-positive animals 6 days after tamoxifen treatment.

Conclusion: As expected, the loss of insulin gene expression in adult animals leads to diabetes. This study sheds light onto the fate of individual beta cells when they lose their ability to express insulin, and reveals the cell fate decisions of other pancreatic cell types when insulin secretory function in the majority of beta cells is lost, in a model that does not induce massive cell killing. These results increase our understanding of the role of insulin in the maintenance of beta cell differentiation and maturity in the adult state, as well as its possible contribution to beta cell death and dedifferentiation in diabetes.

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Reconstruction of alpha-, beta-, and delta-cells into islet architecture upregulates Pdx1 expression through Akt/FoxO1 regulatory pathway

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Background and aims: Islet transplantation has shown promise for treating insulin-dependent diabetes mellitus. However, the execution of this therapy relies largely on the availability of pancreatic tissue, and thus preparation of transplantable islets from cultured pancreatic cells is in high demand. To the best of our knowledge, *in vitro* reconstitution of pancreatic islet from native alpha-, beta-, and delta-cells has not been reported. We have previously generated a bioactive matrix named REP composed elastin-derived domains and integrin-binding Arg-Gly-Asp ligands. Using this matrix, we have very recently constructed islet-like architectures from the mixed culture of alpha-, beta-, and delta-cells from native islets and cell lines. This study aimed to investigate the signaling cascade and regulatory mechanism that promoted the islet cell survival and expression of islet-specific genes during islet reconstitution.

Materials and methods: Islets were isolated from 6-month aged rats and disintegrated into single cell population of alpha-, beta-, and delta-cells by trypsin treatment. Wells in a 24-well plate were treated with 200 μ l of 1 μ M REP matrix and incubated at 37 $^{\circ}$ C for 1 h. After protein precipitation, supernatant PBS solution was pipetted out carefully, and alpha-, beta-, and delta-cells were seeded and cultured for 7 days. On day 1, 3, 5, and 7, the reorganization of islet architecture and mRNA expression were monitored by immunofluorescence microscopy and qRT-PCR, respectively. The protein content and phosphorylation ratio were quantified by Western blot analysis.

Results: Our data showed that both the mRNA and protein abundances of E-cadherin and connexin-36 were much higher in the reconstructed islet than those in the conventional monolayer culture. The inhibition of E-cadherin or connexin-36 expression by siRNA severely limited islet reconstitution, suggesting the involvement of these cell adhesion molecules. Reconstructed islet exhibited higher basal insulin secretory activity, and under glucose-stimulated condition (25 mM glucose) reconstituted islet showed greater glucose-stimulated insulin secretion than the cells in the monolayer culture. Importantly, the mRNA levels of islet-specific genes such as neuroD (Beta2), slc2a2 (Glut2), ins1, ins2, and pdx1 were significantly increased during islet reorganization. In addition, expression of proliferating cell nuclear antigen (PCNA) was greatly upregulated. Furthermore, Akt phosphorylation was also significantly elevated during islet reconstruction. FoxO transcription factors play a crucial role in the regulation of differentiation, proliferation, and survival of pancreatic islet cells. To analyze Akt/FoxO1 regulatory pathway, we inhibited Akt activity during islet reconstitution. The inhibition of Akt by siRNA or MK2206 decreased both the FoxO1 phosphorylation and PCNA and Pdx1 mRNA expression. The inhibition of FoxO1 by AS1842856 caused upregulation of PCNA and Pdx1 mRNA expression and subsequent increase in their protein content. The activation of Akt accompanied by the FoxO1 phosphorylation and cytoplasmic retention of FoxO1, resulting in inactivation of transcriptional repression of Pdx1 gene expression.

Conclusion: We conclude that during islet reconstruction, Akt promotes islet cell survival and islet-specific gene expression through FoxO1 regulation. Supported by: DGIST grants (Project No. 14-NB-01 and 14-BD-06)

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Beta cell mass expansion is impaired in aged rats exposed to 90% pancreatectomy and gastrin treatment

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Background and aims: Aging is a non-modifiable risk factor for the development of diabetes. The contribution of impaired beta cell regeneration to this increased risk remains unknown. In young rats, 90%-pancreatectomy (Px) results in β -cell mass regeneration which is further enhanced with gastrin treatment. The aim of our study was to investigate the β -cell regeneration potential of aged rats using the 90%-Px model and gastrin treatment.

Materials and methods: 1 and 12 month-old Wistar rats underwent 90%-Px and were treated from the day of surgery with [15Leu] gastrin-17 (150 μ g/kg • 12h, Px+G, n=21) or with vehicle (Px+V; n=21). A group of sham-operated rats treated with vehicle was included for each age group (S+V; n=18). Pancreatic remnants were harvested on days 3 and 14 after surgery for morphometric, immunohistochemical and gene expression analysis.

Results: Young Px rats showed increased β -cell mass that was further increased with gastrin treatment. Gene expression and nuclear immunolocalization of nkx6.1 in ductal cells, and the percentage of extra-islet β -cells (indirect markers of β -cell neogenesis) were also increased in gastrin-treated young Px rats. β -cell apoptosis was similar among groups, and β -cell replication and size were similarly increased in gastrin and vehicle-treated young Px rats. In aged rats, β -cell mass was not increased in any of the Px groups, despite the increased β -cell replication and individual β -cell size. Gene expression and nuclear immunolocalization of nkx6.1 in ductal cells, and the percentage of extra-islet β -cells were not increased in aged gastrin- or vehicle-treated Px rats. The dedifferentiation-related transcription factors Neurog3 and Sox9 were significantly upregulated in islet β -cells from aged Px rats.

Conclusion: The potential for compensatory β -cell hyperplasia and hyper trophy is retained in aged rats. In contrast, impaired β -cell neogenesis along with beta cell dedifferentiation may contribute to the limited beta cell regeneration in aged rats.

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Cell surface marker expression and colony formation in islet-depleted pancreatic digests

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Background and aims: Stem cells (SCs) are characterized by self-renewal capacity and pluripotency. Hematopoietic SCs (HSC) are identified by a combination of markers and functional assays. In the adult human pancreas, the existence of SCs is debated. To identify putative stem cells, we screened islet-depleted pancreas digests, a by-product of the islet isolation procedure, for expression of surface markers and epithelial colony-forming potential (CFP).

Materials and methods: Islet-depleted pancreas digests from 18 cadaveric organ donors were stained with antibodies to hematopoietic, mesenchymal (MSC) and epithelial stem cell (EpSC) markers. Cells in the 'side population' (SP) were identified by Hoechst 33342 dye efflux in a BD-LSR-II-W flow cytometer. EpCam, CD133, Hpd1 and Hoechst 33342 alone or in combination were used to separate populations in a FACSAria-C sorter. CFP was evaluated in a 2D assay. Data are presented as mean \pm SEM.

Results: Very few cells expressed HSC markers (CD45 2.7 \pm 0.8 %, CD117 1.2 \pm 0.6 %, CD31 10.8 \pm 0.8 %, CD56 2.8 \pm 1.0 %). The MSC marker CD29 was detected on a majority of cells (97.1 \pm 1.0 %), whereas the other MSC markers CD44 (46.4 \pm 7.8 %), CD34 (34.1 \pm 10.8 %), CD90 (17.2 \pm 4.5 %), CD105 (36.1 \pm 11.1) and CD73 (27.6 \pm 5.3 %) were expressed on fewer cells. Certain EpSC markers, EpCam (75.3 \pm 3.9 %) and CD49f (60.9 \pm 7.1 %), were expressed by a majority of cells whereas others, CD133 (44.6 \pm 7.5 %) and CD26 (20.5 \pm 3.6 %), were expressed by fewer cells. The CFP of pancreas digest cells was however significantly enriched by selection for CD133^{high}SP cells, such that CD133^{high}SP > SP > CD133^{high} > CD133^{low}. The majority of CD133^{high} (61.4 \pm 6.3 %) and SP (58.32 \pm 6.97 %) cells expressed the ductal marker

CA19-9. In the human pancreas, ductal cells express CD133 and comprise a 2-fold increased proportion (3.1 vs. 7.5 %) of the SP.

Conclusion: We identified two markers for the isolation of colony-forming cells. Our findings suggest that ductal cells that efflux Hoechst 33342 and express CD133^{high} are a reservoir of stem cells in the adult human pancreas.

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Highly-efficient MSCs fusion with beta cells results in beta cell-like phenotype

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Background and aims: Mesenchymal stromal cells (MSC) have anti-inflammatory, anti-apoptotic and immunosuppressive effects and they are a potent source for cell therapy. Cell fusion enables rapid generation of functional new reprogrammed cells. In this study, we aimed to establish a fusion protocol of bone marrow derived human MSCs with the rat β -cell line (INS1-E) as well as isolated human pancreatic islets in order to generate functional insulin producing β -MSCs as a cell-based treatment for diabetes.

Materials and methods: Human MSCs were isolated from bone marrow and characterized by colony forming units in culture and flow cytometry for expression of CD73, CD105 and CD90 as well as lack of CD45, CD34 and MHCII. Multipotency was tested by differentiation into the adipogenic and osteogenic lineages. To induce cell fusion, eGFP transfected MSCs with puromycin as selection marker were cultured with either mCherry-INS1E or human dispersed isolated islets and treated with phytohemagglutinin and Polyethylene glycol (PEG).

Results: Of the whole cell population, 44 \pm 10% polykaryons were identified based on polyploidy flow cytometry at 2 days after fusion. 29 \pm 6% of all MSCs were identified as β -MSC heterokaryons based on double positivity for mCherry and eGFP. Six days after fusion and four days after puromycin selection, the β -cell transcription factors Nkx6.1, MafA and PDX1 and important genes for regulating β -cell function insulin, GLUT2 and GCK and insulin content were increased in the β -MSCs from fused human dispersed islets/MSCs as well as INS-1E/MSCs compared to non-fused controls after puromycin selection. Insulin positive β -MSCs also expressed nuclear PDX1.

Conclusion: Our results show an efficient established protocol for fusion of human MSCs and β -cells, which resulted in a β -cell like phenotype; this could be a novel tool for cell-based therapies of diabetes.

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A novel and reliable protocol for human embryonic stem cell differentiation into the definitive endoderm based on dispersed single cells

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Background and aims: The differentiation of embryonic stem (ES) cells into organs derived from the endoderm germ layer, such as liver or pancreas require as initial step the formation of the definitive endoderm (DE). Human ES cell differentiation is usually initiated from colonies, which is the routinely used cell culture procedure. However, differences in colony size, colony number and the potentially altered sensitivity of these cells to media supplements render colonies into a suboptimal starting material. The aim of this study was the development of a reliable and highly efficient protocol for the differentiation of human ES cells into the DE lineage from dispersed single cells and their further differentiation into the pancreatic lineage.

Materials and methods: Three different human ES cell lines were passaged as dispersed single cells and defined cell numbers were subjected to differentiation. Different protocols for the DE induction were tested by combining various concentrations of Wnt3a, CHIR-99021 and Activin A for four days. The expression of the marker genes T, GSC, MIXL1, SOX17 and FOXA2 was analyzed by qPCR, flow cytometry, and immunofluorescent staining (IF). Furthermore, differentiation of these DE cells into the pancreatic lineage with FGF10, retinoic acid, dorsomorphine plus SB-431542 was performed. PDX1- and NGN3-positive cells were quantified by IF.

Results: Differentiation with CHIR-99021 and Activin A for the first 24 h and a subsequent treatment with Activin A alone resulted in the highest numbers of DE committed cells from all three tested cell lines. The expression of the marker genes SOX17 and FOXA2 were significantly increased under these conditions compared to random differentiated cells and to a classical DE differentiation protocol used as positive control. The quantification by IF and flow cytometry of DE committed cells revealed efficiencies ranging from ~70% (Hues4 and HES3) up to >80% (Hues8), a more than 2-fold increase compared to the reference protocol (~33–40%). The ES cells proliferated under this condition resulting in a more than 5-fold increased cell number after four days of differentiation. In addition, the dispersed single cells differentiated into DE were able to further differentiate into PDX1-positive progenitors (~40% after 10 days) and subsequently into NGN3-positive endocrine precursor cells (~10% after 14 days). High expression levels for the marker genes FOXA2, HNF6, MNX1 (HB9), NKX2.2, NKX6.1 and NGN3 in a manner similar to the in vivo pancreatic development were detected proofing the maturation of the pancreatic precursor cells.

Conclusion: This novel protocol was able to differentiate three human ES cell lines in a highly efficient manner into the DE. Therefore only low initial cell numbers and reduced concentrations of expensive growth factors were required without growth limitations. In addition, these cells were able to differentiate further into the pancreatic lineage and endocrine progenitor cells. *Supported by: REBIRTH Cluster of Excellence (DFG)*

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Isolation and characterisation of embryo stem cell-like cells in adult human pancreas

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Background and aims: Within recent years, stem/progenitor cells research has become a very important part of regeneration or differentiation into insulin-producing cells. However, the stem/progenitor cells are extremely rare attendance and expressing location and moment of these stem cells are not clearly demonstrated in adult human pancreatic tissue. Therefore we have identified undifferentiated embryo stem cell-like cells expression in adult human pancreas and isolated for characterization.

Materials and methods: Enriched human exocrine cells are obtained after COBE purification of islet isolation. For islet-depleted pancreatic exocrine cells culture, endocrine cells were sorted out with microbead conjugated PSA-NCAM antibody using magnetic-activated cell sorting (MACS) and purified CA19-9 positive pancreatic ductal cells or non-purified cells were cultured for 6 days. We observed morphology changes and RNA expression pattern of embryo stem cell markers. To identification of stem cells present location, we SSEA-4 positive cell selected from enriched exocrine cell fraction.

Results: Non-purified crud duct cells attached easily and epithelial-like cells extended grow up quickly from primary attached cells but purified ductal cells were showed insufficient growth. Expression of classic stem cell markers: Oct4, c-Myc, Klf4, Nanog, Sox2 and SSEA-4 mRNAs was found in crude duct and purified duct cell fraction. However, these stem cell markers was not detected or weakly expressed in PSA-NCAM negative and CA19-9 negative cell fraction. Classic stem cell mRNA markers were only expressed in SSEA-4 positive cells and SSEA-4 positive cells were detected in pancreatic duct cell by immunocytochemistry.

Conclusion: We characterized and isolated of SSEA-4 positive embryo stem cell-like cells in adult human pancreatic duct and hypothesize that these cells differentiate to insulin-producing cells in adult human pancreas.

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Expression of insulin genes in foetal liver

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Background and aims: Pancreatic beta cells are the major source of insulin in adult mammals. The cells have a limited capacity for regeneration and the destruction causes type 1 diabetes. Recently, a possible permanent cure for diabetes has been explored by using several different approaches, one of them being the transdifferentiation of nonpancreatic cells to insulin-producing cells. Hepatocytes seem to be an ideal target for the production of insulin, because both the liver and ventral pancreas appear to arise from the same cell population located within the embryonic endoderm during embryogenesis. Despite the similarity in development of these two cell types, the expression of the insulin gene in fetal liver has remained elusive because of its pancreatic beta cell-specific expression in adult animals. In the present study, we demonstrate insulin mRNA and immunoreactive cells for insulin in fetal liver and the promoter activity for insulin in fetal hepatocytes.

Materials and methods: Timed pregnant ICR mice were obtained from Japan SLC. On embryonic days 13.5 (E13.5), E16.5 and E18.5, livers were dissected. The livers of newborn mice were dissected at Day0. Livers from adults were dissected from 14-week-old female mice. Gene expression was analyzed by RT-PCR. Immunohistochemical analysis was carried out using antibodies against insulin, proinsulin, processed insulin, glucagon, somatostatin, and pancreatic polypeptide. Promoter activity of mouse Ins1 (-703~+14) and Ins2 (-830~+14) was measured using primary cultured fetal hepatocytes by luciferase assay. Electrophoretic mobility shift assay was carried out using whole-cell extracts of fetal liver. Human fetal (22–40 weeks) and adult liver RNAs, purchased from Clontech, were also analyzed by RT-PCR.

Results: The expression of insulin and transcription factors for insulin is investigated in mouse fetal liver. We detected mRNAs for Ins1 and Ins2 and proinsulin- and mature insulin-positive cells in mouse fetal liver by RT-PCR and immunohistochemistry. Glucagon, somatostatin and pancreatic polypeptide were not expressed throughout development. Mouse Ins2 and Ins1 promoters were transiently activated in mouse fetal hepatocytes of E13.5 and E16.5, respectively. Pancreatic and duodenal homeobox 1 (Pdx1) mRNA was not expressed during development of the liver. In contrast, mRNAs and proteins of neurogenic differentiation (NeuroD)/beta cell E-box transactivator 2 (Beta2) and v-maf musculoaponeurotic fibrosarcoma oncogene homolog (MafA) were almost simultaneously expressed with insulin genes in the liver. Ins2 and Ins1 promoters were activated in hepatoma cells by the transfection of the expression vector for NeuroD/Beta2 alone and by the combination of NeuroD/Beta2 and MafA, respectively. In addition, we also analyzed human fetal and adult liver RNAs and found that mRNAs for insulin, NeuroD/Beta2, and MafA were expressed in human fetal liver.

Conclusion: These results indicate that the expression of NeuroD/Beta2 and MafA is linked temporally with the transcription of insulin gene in fetal liver and suggest the potential usage of fetal hepatocytes to make insulin-producing cells by introducing/inducing transcription factors.

PS 015 Metabolic coupling in insulin secretion

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MICU2: a modulator of stimulus-secretion-coupling in INS-1 832 cells

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Background and aims: In β -cells, glucose-stimulated insulin secretion (GSIS) is tightly coupled to the metabolism of glucose, which ultimately triggers a rise in cytosolic calcium (Ca^{2+}) levels activating the exocytotic machinery. Ca^{2+} is also involved in activation of the more sustained second-phase of insulin secretion. However, the mechanism is less clear. The regulated mitochondrial Ca^{2+} -influx during metabolic activation is critical for GSIS but the function of Ca^{2+}_m is still not fully understood. Mcu and Micu1 are subunits of the Mitochondrial Calcium Uniporter complex (MCU-complex) and have been shown to be important for ATP synthesis and GSIS. Micu2 was recently identified in mouse liver as a part of the MCU-complex functioning as a gate-keeper for Ca^{2+}_m -uptake, similar to Micu1. Our preliminary data show that an expression quantitative trait locus (eQTL) associated with the *MICU2* gene is correlated with β -cell function (HOMA- β) and fasting glucose in humans. The aim of this study was to investigate the role of Micu2 in GSIS and mitochondrial function in the clonal beta-cell line INS-1 832/13.

Materials and methods: *Micu2* was silenced for 72 h by siRNA in INS-1 832/13 cells, and insulin secretion was determined by radioimmunoassay, ATP content by luciferase-dependent luminescence, and lactate release as well as NADH/NAD⁺-ratio by colorimetric assays. Glucose utilization was measured as ³H₂O after stimulation with 5-³H-labeled glucose, Seahorse XF24 was used to measure respiration and metabolite profiling was done by GC/MS.

Results: KD of *Micu2* in INS-1 832/13 cells resulted in a 33% (N=3, p<0.001), 37% (N=3, p<0.01) and 33% (N=3, p<0.05) reduction in insulin secretion compared to control cells at 2.8 mM and 16.7 mM glucose and 10 mM glyceraldehyde, respectively. No alteration in insulin secretion was observed when cells were stimulated by pyruvate or leucine/glutamine. Respiration rate was similarly decreased in *Micu2* KD cells at 2.8 mM and 16.7 mM glucose compared to control cells, while pyruvate-stimulated respiration was unaltered. Interestingly, KD of *Micu2* abolished glucose-stimulated spare respiratory capacity by decreasing the maximal respiration rate by 44% (N=3, p<0.01); this was not observed during pyruvate-fueled respiration. KD of *Micu2* affected neither total cellular ATP content nor lactate secretion while glucose utilization was decreased. A 3.5-fold increase in the glucose-induced rise in NADH/NAD⁺-ratio was observed in *Micu2* KD cells compared to control cells. Metabolite profiling showed a 4-fold (N=3, p<0.05) decrease in glutamate/aspartate ratio upon *Micu2* KD.

Conclusion: *MICU2*, a subunit of the MCU complex, modulated NADH/NAD⁺-shuttle activity rather than dehydrogenase activities in the TCA-cycle since insulin secretion and respiration were decreased only when shuttle-dependent fuels were used. This is corroborated by decreased glucose utilization, unaltered total ATP content and lactate release as well as increased NADH/NAD⁺-ratio upon *Micu2* KD. The decreased glutamate/aspartate-ratio observed points to a perturbation in the malate-aspartate shuttle. Whether *Micu2* acts exclusively in Ca^{2+} activation of the malate-aspartate shuttle or physically interacts with parts of the malate-aspartate shuttle altering its activity directly remains to be resolved.

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Trans-mitochondrial ¹³C-flux analysis supports an important role for non-oxidative metabolism in insulin secretion from human islets

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Background and aims: Non-oxidative mitochondrial metabolism has previously been implicated glucose-stimulated insulin secretion (GSIS). In particular, pyruvate carboxylase (PC) and mitochondrial PEPCK (PEPCK-M) have been strongly associated with GSIS. Recent reports suggested that in

contrast to rodent islets, human islets have relatively little PC flux. The positional flux of [¹³C₆]glucose carbons through each reaction provides important information regarding its relevance to the GSIS without perturbing normal metabolism. Current NMR-based methods are limited by sensitivity and cannot resolve the different anaplerotic fluxes.

Materials and methods: A novel quantitative LC/MS-based technique was developed to track ¹³C-labeled substrates and directly measure positional step-wise metabolic fluxes in INS-1 cells and human islets. Metabolic flux analysis was performed under metabolic steady-state but isotopic non-steady-state conditions. Absolute flux was calculated with the program CWAVE using differential equations to model mass and isotope balance.

Results: Metabolism included oxidative (glycolysis, pyruvate dehydrogenase (PDH) and TCA cycle) and non-oxidative (PC, malic enzyme (ME) and PEPCK-M) reactions. There was significant isotope dilution between sequential steps in glycolysis and TCA consistent with very active metabolic cycling. Similar to oxygen consumption, TCA cycle flux increased incrementally with glucose (1.27±0.02, 2.31±0.06, and 5.02±0.1 fold, P<0.001, going from 2.5 to 5 to 7 to 9 mM glucose) while the contribution of PDH to the TCA cycle (~90%) remained flat. PC flux was nearly evenly divided between ME and PEPCK-M flux, though remarkably, glucose carbons flowed exclusively through PEPCK-M and not ME. PC flux increased with glucose starting at 2% to more than 50% the rate of the TCA cycle (fold increases of 3.73±0.36, 9.89±0.52, and 28.91±2.2 above background, P<0.001) and closely correlated with insulin secretion. PEPCK-M flux increased linearly with insulin secretion. Similar rates of PC relative to PDH fluxes were observed in islets from human donors.

Conclusion: This technique has broad applicability to the study of intracellular metabolism in normal and diabetic human islets as well as other tissues. Quantification of discrete trans-mitochondrial fluxes beta-cells supports a very significant contribution of non-oxidative metabolism to the mechanism of insulin secretion.

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Mechanisms underlying translocation of the cAMP effector protein Epac2 to the beta cell plasma membrane

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Background and aims: The second messenger cAMP promotes insulin secretion by amplifying Ca^{2+} -dependent exocytosis in pancreatic β -cells. Protein kinase A and Epac2, a guanine nucleotide exchange factor for the Rap family of small GTPases, mediate the effects of cAMP. Epac2 translocates to the β -cell plasma membrane in response to glucose and cAMP-elevating agents, but it is not known how the membrane interaction is mediated. Previous studies have shown involvement of the Ras-association domain close to the catalytic domain of the protein. The aim of the present study was to elucidate how different domains in the regulatory region contribute to membrane localization of Epac2 in β -cells.

Materials and methods: MIN6 β -cells were co-transfected with wildtype and mutant versions of Epac2 tagged with the fluorescent proteins GFP and mCherry. Time-lapse total internal reflection fluorescence microscopy was used to monitor association of the reporter proteins with the plasma membrane in single intact or α -toxin-permeabilized cells.

Results: Addition of cAMP to permeabilized β -cells evoked concentration-dependent translocation of Epac2 to the plasma membrane with half-maximal and maximal translocation at 30 μM and >100 μM , respectively. Whereas point mutation of the low-affinity cAMP-binding site (G114E) did not affect cAMP-induced Epac2 translocation, removal of the entire low-affinity cAMP-binding domain (CNB1) reduced the maximal translocation amplitude to 45% of control but increased the cAMP sensitivity (half-maximal translocation at 5.6 μM). After removal of the DEP domain, a protein module often involved in membrane anchoring, the cAMP-induced translocation was reduced by more than 90%. Epac2 translocation was also investigated in intact cells stimulated with 100 μM of the phosphodiesterase inhibitor IBMX or by increase of the glucose concentration from 3 to 11 mM. Both stimuli triggered pronounced translocation of wildtype Epac2 to the plasma membrane. The IBMX-induced translocation after DEP removal was lower (58%) while that of the CNB1 deletion mutant was significantly higher (145%) than control. In contrast, the glucose-induced Epac2 translocation was unaffected by either of the CNB1 or DEP deletions.

Conclusion: cAMP-induced translocation of Epac2 to the plasma membrane involves multiple domains of the protein, including the DEP- and the

low-affinity cAMP-binding domains. The latter domain is not required for translocation, but it extends the range of effective cAMP concentrations and increases the magnitude of the translocation response. Glucose-stimulated Epac2 translocation in β -cells involves additional signals than cAMP elevation alone.

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Does increased mitochondrial pool improves beta cell function? Trying to create super beta cells with PGC-1 α mediated mitochondrial biogenesis

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Background and aims: Understanding diabetes and try to cure it requires defining the regulators of pancreatic beta-cell function. Because beta cells rely on their cellular metabolism, mitochondrial activity and energy production to secrete insulin properly, we tested the idea that augmenting mitochondria in beta cells might improve their insulin secretion capacity, thus generating super beta cells that would be adapted to situations of increased insulin demand. To this aim, we overexpressed in beta cells the transcriptional coregulator PGC-1 α , a potent inducer of mitochondrial biogenesis, and studied both in vivo and in vitro the consequences on insulin secretion.

Materials and methods: To carry the study, we used two models of PGC-1 α overexpression in beta cells: first Min6 cells overexpressing PGC-1 α with an adenovirus and second, transgenic mice with a specific PGC-1 α overexpression in mice using the Tetracycline inducible system. We measured insulin secretion in response to glucose, glucose tolerance in mice, mitochondrial function (cellular respiration, ATP turnover and reactive oxygen species - ROS - production) and assessed the beta-cell energy status by measuring the phosphorylation of the AMP-Kinase.

Results: In Min6 cells, PGC-1 α overexpression increases mitochondrial biogenesis and β -cell respiration but paradoxically, does not increase ATP turnover. PGC-1 α overexpression increases ROS production and leads to the activation of AMPK, suggesting both an oxidative and energetic stress in these cells. Instead of being improved, insulin secretion in response to glucose was blunted when PGC-1 α is overexpressed. Interestingly, rescuing insulin secretion was achieved using a powerful antioxidant (Tempol) to prevent ROS production or using compound C-induced, an inhibitor of AMP-kinase activation. Similar results were obtained on isolated islets from PGC-1 α overexpressing mice: increased ROS production, activated AMPK and blunted insulin secretion.

Conclusion: In beta cells, increasing mitochondria with the use of PGC-1 α overexpression induces both an oxidative stress and an energetic stress. Therefore, strategies to increase insulin function through increased mitochondrial function may not be efficient. Specific mechanisms underlying these defects still need to be identified. In contrast with other metabolic tissues, increased mitochondrial pool using PGC-1 α is not beneficial for insulin production by beta cells.

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Mitochondrial coupling efficiency is swiftly increased by hyperoxia reciprocally to effects by hypoxia

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Background and aims: Previous exposure to hyperoxia was recently shown to negatively affect beta cell function and viability. These findings are important in the context of possible damage to beta cells during hyperoxic treatment in humans, such as in preterm babies, and therefore prompt further studies. The early time course of effects of hyperoxia has not been clarified and may provide information on mechanisms. Accordingly, here we tested for acute effects of hyperoxia on mitochondrial function

Materials and methods: INS-1 derived 832/13 cells were cultured in RPMI medium, usually together with 11 mM glucose. For some experiments INS-1 cells overexpressing uncoupling protein 2 (UCP-2) were used. Oxygen consumption was measured in intact cells by high-resolution respirometry (OROBOROS) before and after sequential administration of oligomycin

(which blocks the production of ATP by ATP synthase), FCCP (which induces respiration that maximises oxidative capacity), rotenone and lastly antimycin. Elevated oxygen concentration (400 μ M) was achieved in one chamber, the other containing normal levels of oxygen (200 μ M).

Results: The presence of hyperoxia decreased the inhibitory effect by oligomycin when the inhibitor was added 15–20 min after the introduction of hyperoxia. The ratio of oligomycin uninhibited respiration in relation to FCCP-induced respiration was increased by 59.4% (from 0.32 ± 0.02 ($n = 6$) at normoxia to 0.51 ± 0.02 ($n = 20$) at hyperoxia, $p < 0.0003$). Similar effects of hyperoxia were seen in INS-1 cells overexpressing UCP-2. The glucose concentration (5.5 or 11 mM) during respirometry did not modify the uncoupling effect of hyperoxia. Opposite effects (decreased ratio oligomycin/FCCP respiration) was seen in cells pre-exposed for 8 h to hypoxia (0.3% oxygen) (0.29 ± 0.01 vs. 0.26 ± 0.02 , $n = 5$, $p < 0.05$).

Conclusion: 1) Beta cell mitochondria swiftly adapt to hyperoxia by increasing un-coupling 2) This effect is not mediated by UCP2, 3) The effect is reciprocal to that exerted by previous hypoxia. Altogether our findings demonstrate swift and relevant adaptability of beta cell mitochondria to differences in the oxygen environment, a feature that should, in the short term at least, promote their survival.

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Inhibition of the malate-aspartate shuttle abolishes glucagon secretion without affecting insulin secretion from mouse pancreatic islets

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Background and aims: Altered secretion of both insulin and glucagon has been implicated in the pathogenesis of Type 2 diabetes (T2D). The main focus of research has been on understanding the impaired insulin secretion from the pancreatic β -cells, leaving the mechanisms controlling glucagon secretion from the pancreatic α -cells largely unresolved.

Materials and methods: The α -cell line, α TC1-6, and β -cell line, INS-1 832/13 were stimulated with glucose, in presence or absence of phenylsuccinate, and pyruvate after which hormone secretion, metabolite levels, glucose utilization, respiration and lactate release were assayed. Key experiments were replicated in isolated mouse islets.

Results: INS-1 832/13 and α TC1-6 cells respectively secreted insulin and glucagon dose dependently in response to glucose. Glycolytic metabolism was similar in the two cell lines; tricarboxylic acid (TCA)-cycle metabolism, respiration and ATP-production were less glucose-responsive in α TC1-6 cells. Hence, a tight coupling of glycolytic and mitochondrial metabolism was observed only in INS-1 832/13 cells. Inhibition of the malate-aspartate shuttle, using phenyl succinate, impacted glucose-provoked ATP production and glucagon secretion from α TC1-6, but not INS-1 832/13 cells. Blocking the malate-aspartate shuttle increased levels of glycerol-3-phosphate only in the INS-1 832/13 cells. Accordingly, expression of components of the glycerolphosphate shuttle relative to expression of the malate-aspartate shuttle was found to be lower in α TC1-6 cells. These results were confirmed in primary mouse islets, where phenyl succinate abrogated secretion of glucagon but not insulin.

Conclusion: Our data suggest that the glycerolphosphate shuttle augments the malate-aspartate shuttle in the β -cell but not so in the α -cell. A suppressed activity of the glycerolphosphate shuttle in the α -cell prevents the α -cell from maintaining a high rate of glycolysis and glucagon secretion at high glucose levels. Importantly, pyruvate- and lactate-provoked glucagon secretion remains unaffected since they are independent of mitochondrial shuttle activity. Consequently, secretion of glucagon becomes more sensitive to fuels available during physical exercise.

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Shared and unique motifs of nutrient sensing in GLP-1 (GLUTag) and insulin (INS-1 832/13) secreting cellsP. Spégel¹, L. Shcherbina², L. Andersson¹, C. Wollheim³, N. Wierup², H. Mulder¹;¹Unit for Molecular Metabolism, ²Unit of Neuroendocrine Cell Biology,³Lund University Diabetes Centre, Malmö, Sweden.

Background and aims: Glucagon-like peptide-1 (GLP-1), secreted from intestinal L-cells, promotes beta-cell proliferation and enhances glucose-stimulated insulin secretion. Despite this important role for GLP-1, much less is known about stimulus-secretion coupling in the L-cell as opposed to the beta-cell. GLP-1 secretion from L-cells has been suggested to be mainly regulated by nutrient up-take rather than metabolism of such fuels. However, it has also been shown that a, as of yet, unknown metabolic signal may potentiate secretion of the hormone. We hypothesized that levels of a potential coupling factor of GLP-1 secretion should rise in nutrient stimulated L-cells in parallel to increased hormone secretion.

Materials and methods: GLUTag and INS-1 832/13 cells were stimulated with a range of different secretagogues in presence and absence of various pharmacological inhibitors. Nutrient-provoked hormone secretion was assessed, in addition to profiling of alterations in metabolite levels by gas chromatography/mass spectrometry.

Results: Both glucose and glutamine stimulated TCA-cycle metabolism (3-9-fold, $p<0.05$) and provoked GLP-1 (1.3-fold, $p<0.001$, glucose; 1.7-fold, $p<0.001$, glutamine) secretion from GLUTag cells; only glucose evoked secretion of insulin (5-fold, $p<0.001$) from INS-1 832/13-cells. Both stimuli resulted in a rise in intracellular glutamate levels in GLUTag (5-fold, $p<0.001$, glucose; 1.7-fold, $p<0.001$, glutamine) and INS-1 832/13 (1.3-fold, $p<0.01$, glucose; 30-fold, $p<0.001$, glutamine). Addition of leucine, activating glutamate dehydrogenase, did not affect GLP-1 secretion from GLUTag cells while robustly releasing insulin from INS-1 832/13 cells (5.2-fold, $p<0.05$). The membrane permeable glutamate analogue dimethyl-glutamate yielded similar levels of GLP-1 secretion (1.6-fold, $p<0.001$) and intracellular glutamate levels (23-fold, $p<0.05$) as glutamine in the GLUTag cells. Addition of leucine did not affect dimethyl-glutamate stimulated GLP-1 secretion, but yielded similar levels of insulin secretion (5-fold, $p<0.05$) from INS-1 832/13 cells as the combination of glutamine and leucine. The membrane permeable pyruvate analogue methyl-pyruvate provoked neither GLP-1 secretion nor elevated glutamate levels in GLUTag cells, despite a strong effect on TCA-cycle metabolism (5-15-fold, $p<0.05$). The same compound elicited a significant increase in TCA-cycle metabolism (4-16-fold, $p<0.01$) and insulin secretion (3-fold, $p<0.01$) similar to pyruvate in the monocarboxylate transporter-1 expressing INS-1 832/13 cells; glutamate levels were unaltered. Inhibition of the oxoglutarate transporter with phenyl-succinate affected neither GLP-1 secretion nor glutamate levels in GLUTag cells, but decreased insulin secretion (-34%, $p<0.05$) and glutamate levels (-25%, $p<0.05$) in INS-1 832/13 cells.

Conclusion: Our findings show that metabolic coupling differs between L- and the beta-cells. Specifically, involvement and activity of glutamate dehydrogenase, mitochondrial metabolism and shuttles differed. Importantly, non-electrogenic elevation of glutamate is a signal for GLP-1 secretion, suggesting that glutamate may be a coupling factor in both glucose- and glutamine-stimulated GLP-1 secretion.

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Regulation of Glucose-Stimulated Insulin Secretion (GSIS) by miR-130a/b and miR-152 via pyruvate dehydrogenase E1 component, alpha subunit PDHA1

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Background and aims: MicroRNAs (miRNAs) are small RNAs that regulate genes at the post-transcription level and have been implicated in beta cell dysfunction. We have previously identified miR-130a/b and miR-152 to be up-regulated in the pancreatic islets of the non-obese type-2 diabetes (T2D) rat model Goto-Kakizaki (GK). Here we aim to investigate the contribution of these miRNAs in glucose-stimulated insulin secretion (GSIS) and to dissect their molecular functions using insulin-secreting cells.

Materials and methods: We cultured the “glucose-responsive”, INS-1 832/13 and “glucose-unresponsive”, INS-1 832/2 cells at low (2.8 mM) and high (16.7 mM) glucose concentrations for 1h. The expression levels of miR-130a/b and miR-152 were measured by qPCR. MiRNAs were over-expressed by transfecting mature miRNA sequences of miR-130a/b and miR-152. The effect on targets predicted by TargetScan was evaluated both on mRNA (qPCR) and protein levels (western blot). GSIS assays (at 2.8 mM and 16.7 mM glucose) were performed and the released insulin was measured using radioimmunoassay. Oxygen consumption rates were measured using extracellular flux analyzer, XF24 (Seahorse Bioscience).

Results: Similar to previous findings in GK islets, we found upregulation of miR-130a/b and miR-152 in the glucose-unresponsive INS-1 832/2 cells compared to INS-1 832/13 cells. At high glucose concentrations, the expression of the miR-130a, miR-130b and miR-152 decreased in INS-1 832/13 by 80 % ($n=3$, $p<0.05$), 75 % ($n=3$, $p<0.001$) and 60 % ($n=3$, $p<0.05$) respectively but not in INS-1 832/2. Perturbing the miRNA levels in INS-1 832/13 cells by over-expression of mature miR-130a and miR-152 resulted in reduced insulin secretion by 30% at 16.7 mM glucose ($n=3$; $p<0.01$) and insulin content by 70% ($n=3$; $p<0.05$). We also observed decreased oxygen consumption rate after miR-130b over-expression. The putative target of the miRNAs, pyruvate dehydrogenase E1 component, alpha subunit (PDHA1) was reduced by 40% at both mRNA ($n=3$, $p<0.05$) and protein level ($n=3$; $p<0.05$) upon miRNA overexpression. Finally we showed that PDHA1 knock-down by siRNA reduced glucose-stimulated insulin secretion by 40% ($n=3$; $p<0.05$).

Conclusion: We utilized clonal beta cells with disparate GSIS phenotypes to study the contribution of miRNAs in the beta cells. Perturbing the levels of miR-130a/b and miR-152 in the glucose-responsive cell line resulted in defective beta cell phenotype such as reduced insulin secretion, reduced insulin content and decreased oxygen consumption rate. Consequently, deregulation of downstream miRNA targets, such as the PDHA1, a component of pyruvate dehydrogenase complex responsible for metabolic coupling in insulin beta cells, potentially contributes in beta cell dysfunction.

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PS 016 Modulation of islet function through cell surface receptors

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Differential action of GLP-1 and GIP on human pancreatic islet function and viability

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Background and aims: Gastric inhibitory peptide (GIP) and glucagon-like peptide 1 (GLP-1) have been studied intensively in various animal models. Physiological studies of these incretins in human pancreatic islets are still lacking. Here, we have assessed the direct effects of GIP and GLP-1 on islets from non-diabetic and diabetic adult human donors.

Materials and methods: We investigated how the incretins affect glucose induced human islet cell activity by MTS assay. Insulin secretion from islets of 61 human donors was measured at 1 and 16.7 mM glucose. To verify various gene expression, RNA-sequencing was performed on human islets.

Results: When challenged by high glucose (20 mM, 72 hr), the number of metabolically active human islet cells decreased (60% decrease vs. 5.5 mM glucose, $p < 0.001$), which can be partially rescued by GLP-1 analogue exendin-4 (40% decrease vs. 5.5 mM glucose, $p < 0.001$), and completely reversed by GIP. In islets from diabetic donors, there was a significant decrease in numbers of metabolically active cells, and further reduced at high glucose (60% decrease, $p < 0.001$). GIP completely reversed the effect of high glucose on reducing the number of metabolically active cells, while exendin-4 showed no effect. Next, we measured effect of the incretins on insulin secretion in islets from 61 donors at basal glucose (1 mM) and glucose stimulated insulin secretion (GSIS, 16.7 mM). The donors could be subdivided into two groups based on their response to glucose and incretins. In group 1 (37 donors), islets showed low basal (0.12 ± 0.01 ng/islet/hr) and GSIS (0.44 ± 0.03 ng/islet/hr) and responded well to GLP-1 (55% increase, $p < 0.01$), but not to GIP. Group 2 (24 donors) showed high basal (0.47 ± 0.05 ng/islet/hr) and GSIS (1.59 ± 0.14 ng/islet/hr), had no further stimulatory effect of GLP-1 or GIP on GSIS. RNA-sequencing on islets showed that group 1 has higher mRNA expression of glucose transporter 1 gene (SLC2A1, 5.5%, $p < 0.05$) and lower expression of glucose transporter 2 gene (SLC2A2, 26.7%, $p < 0.01$) compared to group 2.

Conclusion: In conclusion, this work provides a first description of incretin effects in human pancreatic islets. We also showed that islet preparations from cadaver donors display different responses to glucose and incretins.

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Isolation of target genes of liraglutide, a GLP-1 receptor agonist, in pancreatic islets by using nano-LC-MS/MS system

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Background and aims: Activation of GLP-1 receptor potentiates insulin secretion and beta cell proliferation in endocrine pancreas. However, the precise mechanisms by which GLP-1 receptor agonist regulate signaling pathway involving insulin secretion and beta cell proliferation remain elusive. In the present work, we performed quantitative comparative proteome analysis of liraglutide-treated mouse islets by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify novel target genes of GLP-1 receptor activation.

Materials and methods: Isolated islets from 8 to 10-week old C57BL/6 male mice were incubated at 5.6 mM glucose in the presence or absence of liraglutide at a concentration of 1000 nM. More than 50 islets were lysed with urea, digested with trypsin, and desalted. The extracts were subjected to nano-LC-MS/MS analysis by using LTQ Orbitrap Velos. The data were analyzed using Progenesis LC-MS software and Databases for Annotation, Visualization and Integrated Discovery (DAVID) software.

Results: We first confirmed that the treatment of liraglutide significantly increased proliferative BrdU-incorporated cells in isolated islets compared with vehicle control ($p < 0.05$). A total of 26225 proteins were identified from 1 μ g of protein extracts in islets by LC-MS/MS. Among them, the expression levels of 1012 proteins were increased, and those of 809 proteins were decreased in the presence of liraglutide. Analysis based on Gene Ontology

(GO) categories revealed that genes up-regulated by liraglutide were linked to the biological processes of translation, vesicle-mediated transport, glycolysis, and glucose metabolic process (enrichment score ≥ 1.3). There were 19 proteins whose expressions were significantly elevated more than 2-fold by the treatment with liraglutide (anova $p < 0.05$), including such as Carboxypeptidase A1, Protein disulfide-isomerase A2 (Pdia2), Glycerol-3-phosphate dehydrogenase 1-like protein, and Lithostathine-1 (Reg1). We here focused on Pdia2 and Reg1, since Pdia2 is supposed to play a role as a molecular chaperone and Reg1 is suggested to be involved in β cell regeneration or growth. We analyzed mRNA expression of Pdia2 and Reg1 in islets at the concentration of 5.6 mM glucose, liraglutide-treated islets showed modestly higher expression than islets of vehicle control (1.28 fold and 1.39 fold, respectively). The mRNA expression levels of these proteins were enhanced in the presence of 11.1 mM glucose (Pdia2: 7.8-fold, $p = 0.058$ and Reg1: 9.4-fold, $p = 0.061$). Whereas there were 12 proteins that were decreased less than 0.5-fold by liraglutide, Caveolin-1, a protein which is the principal structural protein of caveolae membranes, only showed significant decrease by the treatment with liraglutide (anova $p < 0.05$).

Conclusion: These results suggested that liraglutide stimulated islet cell proliferation and altered the expression levels of proteins such as Pdia2, Reg1 and Caveolin-1 in vitro. Pdia2 and Reg1 are suggested to be involved in proliferation and regeneration of β cells. Caveolin-1 is reportedly related to the regulation of exocytosis and insulin receptor-mediated signaling. GO analysis showed that the target molecules of liraglutide were related to the functions involved in glucose metabolism, and the glucose signal in islets potentiated the expression of Pdia2 and Reg1 induced by liraglutide. In conclusion, Pdia2 and Reg1 are novel targets of liraglutide and may be involved in the proliferation signal in islets.

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Dipeptidyl peptidase 4 is expressed in pancreatic islets in a species specific manner, and its activity is increased by high glucose in isolated mouse islets

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Background and aims: Functional glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic peptide were both discovered in pancreatic α cells recently, indicating a local incretin system within islets. However, whether dipeptidyl peptidase-4 (DPP-4), an indispensable part of the system, is expressed in islets and therefore may be involved in the local regulation of islet function remains uncertain. This study examined the islet distribution of DPP-4 in different species, as well as the possible regulating factors of its activity.

Materials and methods: Co-localization of DPP-4 and insulin or glucagon was examined with immunofluorescent staining in pancreatic sections of humans, pigs, C57BL/6 mice and Sprague Dawley rats. Isolated islets from wild type mice were cultured in different conditions for 72 hours in RPMI medium (with 0.1% bovine serum albumin, 100U/ml penicillin and 100 μ g/ml streptomycin) with the medium changed every day. DPP-4 activity of the islets was then measured using Gly-Pro-pNitroaniline as the substrate, and Nitroaniline as the standard. The examined conditions included different levels of glucose and 5.6mmol/L glucose plus 10nmol/L insulin or 100nmol/L GLP-1. DPP-4 activity was also analyzed in islets from GLP-1 receptor knock-out mice (GLPR^{-/-}) and double incretin receptor knockout mice (DIRKO).

Results: DPP-4 fluorescent signals were readily observed in islets of all species. A pronounced species difference was observed, however. In human and pig islets, DPP-4 was expressed exclusively in α cells, with nearly no expression in β cells. In contrast, in mouse or rat islets, DPP-4 was expressed in a β cell dominant manner although low degree of expression was discovered in α cells (fig.1). DPP-4 activity in isolated mouse islets was gradually increased by high glucose overtime, and the differences between 5.6 mmol/L group (3.6 ± 1.2 pmol/min/islet) and the high glucose groups was statistically significant after 72 hours of culture (6.3 ± 1.1 , 6.7 ± 2.5 , and 7.3 ± 1.5 pmol/min/islet for 11.1, 16.7, and 33.3mmol/L, respectively, $P < 0.05$ vs. 5.6mmol/L for all groups, $n = 5$). There was no significant effect of 10nmol/L insulin or 100nmol/L GLP-1 on DPP-4 activity compared with control (5.3 ± 1.5 , 4.9 ± 1.0 and 5.7 ± 1.1 pmol/min/islet respectively, $P = 0.72$ $n = 3$). Lack of impact of GLP-1 on intraislet DPP-4 activity was further confirmed by the result that DPP-4 activity was similar in wild type mice, GLPR^{-/-} mice and DIRKO mice (6.5 ± 2.1 , 6.9 ± 1.6 and 6.5 ± 0.7 pmol/min/islet respectively, $P = 0.96$, $n = 3$).

Conclusion: Active DPP-4 is expressed in islets in a species-specific manner. Local DPP-4 activity is directly increased by high glucose per se rather than via insulin or GLP-1 indirectly. The underlying regulatory mechanism of DPP-4 activity and its clinical implications, as well as the importance of the species difference, remain to be further investigated.

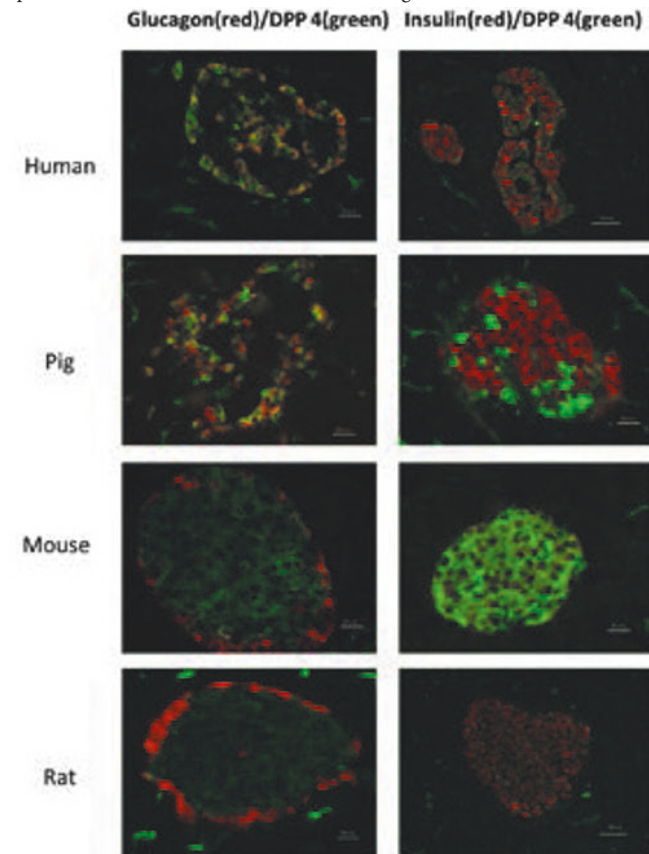


Figure 1. Co-localization of DPP (green) / glucagon(red) as well as DPP (green) / insulin (red) in human, pig, mouse and rat islets.

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Defective compensatory insulin secretion causes HFD-induced glucose intolerance in mice with point mutation in free fatty acid receptor 1

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Background and aims: The fatty acid receptor 1 (FFAR1/GPR40) mediates the fatty acid-dependent augmentation of glucose-induced insulin secretion (GIIS) in pancreatic beta-cells. This feature led to the generation of specific FFAR1-agonists for the treatment of hyperglycemia. Intriguingly, *Ffar1*^{-/-} mice were protected against liver steatosis, suggesting that antagonists of *FFAR1* could have beneficial effects on metabolism. To further understand the impact of *FFAR1* on the regulation of glucose homeostasis and insulin disposal, mice carrying point mutations in *Ffar1* were generated.

Materials and methods: 16,800 archived sperm samples were screened for N-ethyl-N-nitrosourea-induced point mutations in *Ffar1*. Mice were generated on C3HeB/FeJ background using three different sperm samples, each carrying one missense mutation in *Ffar1*. Heterozygous and wild-type littermates were fed chow (CD) or high fat diet (HFD) for 8 weeks. Thereafter, glucose tolerance and insulin sensitivity as well as total pancreatic insulin

content were assessed. Insulin secretion was measured in isolated islets of homozygous mice.

Results: Three mice strains, two with single point mutations in the extracellular (T146S and R258W) and one in the intracellular domain (L106P) of the receptor showed normal development, behavior and growth. HFD-induced insulin resistance and male mice deviated from normal glucose tolerance, although the pancreatic insulin content was almost doubled in all the mice regardless of genotype. Most interestingly, heterozygous mice carrying the T146S-mutation in *FFAR1* developed significant glucose intolerance despite the adaptive increase in insulin production. Isolated islets from T146S homozygous animals showed impaired insulin secretion in response to the *FFAR1* agonist TUG-469.

Conclusion: Proper activation of *FFAR1* is necessary for insulin hypersecretion in the insulin resistant state, in order to protect from HFD-induced glucose intolerance, but *FFAR1* receptor signaling may not contribute to the adaptive increase in pancreatic insulin synthesis.

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Stimulation of free fatty acid receptor 1 reduces thioredoxin interacting protein and exhibits anti-apoptotic properties in insulin secreting cells

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Background and aims: Multiple observations suggest that glucose-dependent stimulation of thioredoxin interacting protein (TxNIP) expression contributes to glucotoxicity. TxNIP inhibits thioredoxin thereby increasing the mitochondrial oxidative stress. Glucose upregulates TxNIP through a ChREBP-dependent transcriptional activation. ChREBP is negatively regulated by FOXO1. Palmitic acid activates FOXO1 through PKCδ and is known to downregulate TxNIP, inspite of inducing apoptosis under glucolipotoxic conditions. Since palmitic acid also acts as a ligand for Free fatty acid receptor 1 (FFAR1), TxNIP downregulation could occur via metabolism or receptor activation. Recently we observed that *FFAR1* agonists exhibit anti-apoptotic properties under glucolipotoxic conditions. Present study investigates the underlying mechanism of the putative protective effects of *FFAR1* agonists.

Materials and methods: Human islets received from the European Centers of Islet Transplantation, mouse islets isolated from C57BL/6 and *Ffar1*^{-/-} mice and INS-1E cells were treated either with palmitic acid (50–600 μmol/l) or the *FFAR1* agonist and antagonist (TUG-469 and TUG 761 respectively, 0.1–10 μmol/l). Changes in expression were analysed by qRT-PCR and western blot. Protein expression was downregulated by siRNA techniques.

Results: In human and mouse islets, as well as INS-1E cells, palmitic acid (600 μmol/l) reduced TxNIP mRNA by more than 50% under 11 mmol/l glucose. This effect was also observed in *Ffar1*^{-/-} mouse islets. Downregulation of PKCδ reversed the effect of palmitic acid on TxNIP, while, INS-1E cells overexpressing PKCδ and displaying high levels of nuclear FOXO1, had extremely low levels of TxNIP. In INS-1E cells, *FFAR1* agonist (3–10 μmol/l) reduced TxNIP by more than 60% whereas the antagonist (10 μmol/l) doubled it. In contrast to palmitic acid, the *FFAR1* agonist (10 μmol/l) downregulated TxNIP in a *FFAR1* dependent manner. However, downregulation of either PKCδ or FOXO1 could not reverse the effects of the ligands.

Conclusion: The observations suggest that inhibition of TxNIP could be mediated by two distinct pathways: one pathway involves PKCδ dependent nuclear accumulation of FOXO1, stimulated by high concentrations of palmitic acid, while the other involves *FFAR1* and does not seem to involve PKCδ and FOXO1, however, it may contribute to the anti-apoptotic property of *FFAR1*.
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Expression and function of an omega-3 fatty acid receptor GPR120 in Islets of Langerhans

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Background and aims: GPR120 and GPR40 are both activated by medium and long chain fatty acids, although they share only 10% amino acid homology. Several previous studies have demonstrated important effects on islet

function of GPR40 activation, but much less are known about the expression and function of GPR120 in islets, although it has been implicated in other metabolic processes. The current study investigated the expression of GPR120 in mouse and human islets, and measured the effects of a GPR120 agonist on islet function.

Materials and methods: Expression of GPR40 and GPR120 mRNAs was assessed by quantitative RT-PCR. Fluorescence immunohistochemistry was performed to localise GPR120 protein expression in mouse and human pancreas. Insulin, glucagon and somatostatin secretion were quantified by radioimmunoassay. Caspase 3/7 activities were measured to determine apoptotic responses to a mixed cytokine challenge (50 U/ml IL-1 β , 1000 U/ml TNF- α , 1000 U/ml INF- γ).

Results: Both human and mouse islets expressed GPR120 mRNA, which was 6 times higher than GPR40 mRNA expression in mouse islets (data are expressed relative to β -actin mRNA levels; GPR120: 0.4 ± 0.08 ; GPR40: 0.07 ± 0.01 ; $n=6$; $*p<0.05$). In mouse islets co-localisation of GPR120 immunoreactivity was detected in both insulin-expressing β -cells and glucagon-expressing α -cells, but not with somatostatin-expressing δ -cells. In contrast, GPR120 immunoreactivity co-localized only with β -cells in human islets. Cytokine-induced apoptosis was significantly reduced in the presence of a selective GPR120 agonist, GAIII (luminescence: control: $50,525 \pm 2,473$ 100nM GAIII: $43,755 \pm 2,054$ 1 μ M: $35,491 \pm 2,828$ 10 μ M: $38,966 \pm 2,519$; $n=8$, $P<0.01$). GAIII had no effect on basal (2mM glucose) insulin secretion but induced a significant potentiation of glucose-stimulated insulin secretion from mouse islets (20 mM glucose: 2.02 ± 0.7 ; +1 μ M GAIII: 4.18 ± 1.22 ng/islet/30min; $n=8$, $P<0.05$). In contrast, GAIII (1 μ M) had no effect on basal or stimulated glucagon and somatostatin secretion ($p>0.2$). Perfusion experiments using mouse islets confirmed GAIII enhanced glucose-induced (20mM) insulin secretion and that this effect was sustainable for the duration of exposure to GAIII. The effects of GAIII to stimulate insulin secretion were reduced in the presence of a PLC inhibitor, U-73122, but not by the PKC inhibitor Go6976 (peak stimulation: 1 μ M GAIII: $203 \pm 42\%$ of 20mM glucose alone; 1 μ M GAIII+1 μ M Go6976: 218 ± 5 ($p>0.2$); 1 μ M GAIII+1 μ M U-73122: $109 \pm 5\%$ ($p<0.01$): $n=3$). **Conclusion:** The medium and long chain free fatty acid receptors GPR120 and GPR40 are expressed in both mouse and human islets. GPR120 expression was identified in β -cells and α -cells in mouse islets, but only in β -cells in human islets. GPR120 activation by GAIII induced a sustained potentiation of glucose-induced insulin release, but had no effect on basal insulin release, nor on basal or stimulated glucagon and somatostatin secretion. Furthermore, GAIII protected mouse islets against cytokine-induced apoptosis. These observations indicate that GPR120 is expressed by islets where it may offer a therapeutic target for regulating insulin secretion and islet cell apoptosis.

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Expression of GPR56 and collagen III in islets: a role in modulating insulin secretion

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Background and aims: GPR56 is a member of the adhesion class of G-protein coupled receptors (GPCRs) and we have identified that it is one of the most highly expressed GPCRs in human islets. Collagen III is its primary agonist in the developing brain where it is found to play a significant role in formation of the cortex, and CD81, a scaffolding protein, couples GPR56 with Gq to form a stable complex in the brain. GPR56 has been associated with inhibition of apoptosis in leukaemia cells. The role of GPR56 in islets is not known, so the aim of this study was to investigate its expression and function in islets.

Materials and methods: GPR56, collagen III and CD81 expression were investigated in mouse and human islets by RT-PCR, Western blotting and immunohistochemistry. Insulin secretion was quantified by radioimmunoassay after acute (1 hour) or chronic (3 days) exposure to collagen III. Islet insulin content after chronic exposure was assayed by acid ethanol extraction. Apoptosis induced by exposure of mouse islets to a cytokine cocktail was quantified by the Caspase-Glo luminescence assay.

Results: GPR56 and CD81 mRNAs were detected by RT-PCR in MIN6 β -cells and in mouse and human islets, while mRNA encoding collagen III was detected in islets but not in MIN6 β -cells. Islet GPR56 expression was confirmed by detection of a 65kDa immunoreactive protein and immunohistochemistry revealed that collagen III was present in islet capillaries but

not in endocrine cells. Isolated mouse islets exposed to collagen III for 1 hour showed significant reduction in insulin secretion at 2mM glucose (0.41 ± 0.06 ng/islet/h, + 100nM collagen III: 0.11 ± 0.02 , $n=6$, $P<0.01$) and 20mM glucose-induced insulin secretion was also inhibited (4.05 ± 0.74 , + 100nM collagen III: 1.64 ± 0.32 , $n=6$, $P<0.01$). Conversely, islets incubated with 100nM collagen III for 3 days exhibited enhanced insulin secretion at both basal and stimulatory glucose concentrations (2mM glucose: 0.26 ± 0.04 , +100nM collagen III: 0.83 ± 0.18 ; 20mM glucose: 6.33 ± 0.63 , +100nM collagen III: 13.30 ± 1.18 ; $n=6-8$, $P<0.01$). There was no effect of 3 days exposure of islets to collagen III on insulin content ($P>0.2$). Measurements of caspase 3/7 activities indicated that collagen III did not provide protection against apoptosis, either basally (luminescence units: $14,307 \pm 1,830$; + 100nM collagen III: $10,410 \pm 1,577$, $n=8$, $P>0.1$), nor in response to exposure to a cytokine cocktail (luminescence units: $46,746 \pm 2,038$; +100nM collagen III: $53,452 \pm 2,671$, $n=8$, $P>0.2$). **Conclusion:** GPR56 is expressed by mouse and human islets and collagen III is localised to islet blood vessels, suggesting that GPR56 may be regulated in a paracrine manner by local interaction with its activating ligand. Exogenous collagen III does not significantly affect islet apoptosis, but prolonged treatment with collagen III enhances insulin output. Given the proximity of collagen III to islets in the pancreas it is likely that the chronic stimulatory effects are physiologically relevant and GPR56 may be a mechanism through which the islet vasculature can regulate β -cell function.

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The mitogenic effect of Gas6 on pancreatic beta cells is mediated via the receptor tyrosine kinase Axl

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Background and aims: The pancreatic β -cell mass adapts through life to cope with changing metabolic requirements. Thus, the β -cell mass expands in situations with increased insulin requirements such as the perinatal period and during pregnancy. Growth arrest specific protein 6 (Gas6) is a ligand for the TAM family of receptor tyrosine kinases, comprising Tyro3, Axl and Mer, with the highest affinity for Axl. We have shown that Gas6 induces proliferation of β -cells in perinatal rats in an age-dependent manner and that β -cells express Axl. Whether the mitogenic effect of Gas6 on β -cells is mediated via Axl is unknown. Besides, Gas6 could potentially affect the β -cell mass in other situations in life where the β -cell mass expands. The aims of the current study were to 1) identify if Gas6 induce β -cell proliferation via Gas6-Axl signaling, 2) identify the cell signaling pathway involved, and 3) determine if plasma levels of Gas6 and sAxl (a soluble form of Axl) is regulated in the third trimester of pregnancy where an expansion of the β -cell mass occurs.

Materials and methods: The effect of BGB324 (small molecule inhibitor of Axl) on Gas6-induced β -cell proliferation and cell signaling, studied with a phospho-kinase array and western blotting with specific antibodies, was examined in INS1E cells and primary islets of Langerhans isolated from neonatal rats. Blood samples for measurement of Gas6, sAxl, leptin, adiponin and TNF- α were previously collected from healthy pregnant women ($n=23$), recruited at the start of pregnancy, at median 14 (range 12–16, $n=9$), 27 (25–29, $n=9$) and 33 weeks (31–35, $n=23$) of pregnancy. Thirteen healthy women served as a non-pregnant control group.

Results: Gas6 increased proliferation of INS1E cells and neonatal rat β -cells 25% and 60% ($p<0.05$), respectively, and this effect was blunted by BGB324. After 30 min. stimulation Gas6 induced a transient increase in phosphorylation of Akt protein (pS473) in neonatal islets, but not in INS1E cells. The rise in p-Akt was blunted by BGB324. There was no difference in plasma levels of Gas6 ($p=0.76$) or sAxl ($p=0.90$) at 14, 27 or 33 gestational weeks or compared to non-pregnant women. In the third trimester (33 weeks) sAxl plasma levels correlated positively with plasma leptin ($R^2=0.37$; $p=0.035$) and plasma adiponin ($R^2=0.39$; $p=0.031$) and tended to correlate inversely with plasma TNF- α ($R^2=0.30$; $p=0.064$).

Conclusion: The mitogenic effect of Gas6 on pancreatic β -cells is mediated via Gas6-Axl signaling. The PI3K/Akt pathway is possibly involved in this response. The plasma levels of Gas6 and sAxl are not changed through pregnancy or compared to non-pregnant women, suggesting that circulatory Gas6/sAxl is not involved in the expansion of the β -cell mass in pregnancy. However, the positive correlations of sAxl with leptin and adiponin and nega-

tive correlation with TNF- α , associated with adipose tissue mass, insulin resistance, and inflammation, could reflect a beneficial anti-inflammatory role of sAxI. Gas6/Axl has been reported to repress TNF- α expression in various cell types including β -cells. Further studies are needed to understand the biological importance of the correlations.

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Persephin: a new player in beta cell proliferation

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Background and aims: Defects in beta cell proliferation and survival are known to contribute to the development of Type 2 diabetes. Previously glial cell line derived neurotrophic factor (GDNF) has been shown to improve beta cell proliferation and survival. GDNF is a member of the GDNF family ligands (GFLs) which also includes artemin, neurturin and persephin. These molecules are all potent neurotrophic factors which preferentially signal via GDNF family receptor α 1-4 (GFR α 1-4) respectively. Pancreatic beta cells share a range of properties with neurons, such as expression of glutamate receptors and maintenance of membrane electrical properties which are critical for the development of action potentials involved in the release of insulin. Expression of GDNF family receptors extends these similarities. Indeed, GFLs are essential for the appropriate innervation of the developing pancreas, these nerves are associated with Schwann cells which are known to release GFLs. Currently the effects of GFLs on pancreatic beta cells are unknown and we aim to elucidate their role using the murine beta cell line, MIN6c4, as a model.

Materials and methods: Quantitative RT-PCR and western blotting were used to measure mRNA and protein expression respectively. Click- iTTM EdU kit was used to assess cell proliferation. Trypan blue was used to assess cell viability.

Results: Expression of GFR α 1-4 mRNA was detected in MIN6c4 cells, mouse islets and human islets (n=3, except human islets where n=2). GFR α 4 displayed at least 10 fold lower expression than all other receptors (Table 1). However protein expression of these receptors did not correlate with the mRNA levels. In MIN6c4 cell lysates both GFR α 1 and GFR α 4 protein was detected, but the truncated form (isoform 2) of GFR α 2 was more abundantly expressed than the complete receptor and GFR α 3 was undetected. Following 16hr serum starvation, MIN6c4 cells were incubated for 6 hrs with 100ng/ml of each GFL. Both GDNF and persephin significantly increased proliferation, whereas neurturin and artemin had no effect (fold increase compared to untreated (mean \pm sem); GDNF: 1.8 ± 0.21 , p<0.01, persephin: 1.6 ± 0.17 p<0.05, neurturin: 1.2 ± 0.16 and artemin: 1.2 ± 0.14 . One way ANOVA, post hoc Dunnett's test). In contrast after 1hr pretreatment with 100ng/ml of each GFL followed by 24 hr incubation with 0.5mmol/l palmitate to induce cell death, no protective effects were observed.

Conclusion: The lack of protein expression of GFR α 2 and GFR α 3 in MIN6c4 cells supports the lack of proliferative effects observed after treatment with their respective ligands artemin and neurturin. However the expression of GFR α 4 protein, despite low levels of mRNA expression, correlates with the ability of persephin to promote proliferation and suggests that, in addition to GDNF, another member of this family, is able to promote beta cell growth, thus identifying an additional potential therapeutic target.

Table 1.

Primer	GFR α 1-4 mRNA expression					
	MIN6c4		Mouse Islet		Human Islet	
	dCT (a.u)	s.e.m	dCT (a.u)	s.e.m	dCT (a.u)	s.e.m
GFR α 1	0.00373	0.00318	0.09391	0.01807	0.27128	0.27128
GFR α 2	0.00781	0.00543	0.03347	0.01630	34.55132	34.20306
GFR α 3	0.22172	0.10529	0.02459	0.01171	0.37586	0.33261
GFR α 4	0.00024	0.00015	0.00852	0.00616	0.03652	0.03652

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Overexpression of angiotensin II type 2 receptor gene induces cell apoptosis and dysfunction of insulin secretion in rat insulinoma (INS-1) cells

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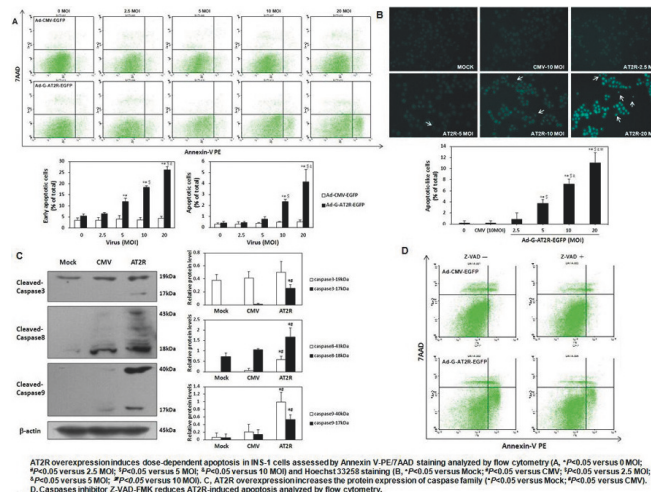
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Background and aims: AngiotensinII (AngII) exerts its effects by activating its receptors, primarily type 1 (AT1R) and type 2 receptor (AT2R), however there is little known about the effects of AT2R on the function of pancreatic islet beta cells.

Materials and methods: To identify the precise role of AT2R in pancreatic islet beta cell, INS-1 cells were transfected with a recombinant adenoviral vector expressing AT2R (Ad-G-AT2R-EGFP). After transfection, the cell apoptosis was detected by Annexin V and Hoechst staining; cell viability was determined by MTT assay and crystal violet staining; glucose-stimulated insulin secretion (GSIS) and genes expression of components of classic GSIS pathway and the apoptotic pathway were examined.

Results: We found that AT2R overexpression alone induced cell apoptosis and suppressed cell viability in a dose- and time-dependent manner. These effects did not require exogenous AngII. Meanwhile, increased expression of AT2R was associated with decreased GSIS, and downregulation of insulin, pancreatic and duodenal homeobox (PDX)-1, glucose transporter (GLUT) 2 and glucokinase (GCK) mRNA expression in a dose-dependent manner. Moreover, overexpression of AT2R markedly increased cleaved caspase-3, caspase-8, and caspase-9 protein expression, but decreased Bcl-2, phospho-Akt, and phospho-ERK protein expression. AT2R-induced cell apoptosis could be blocked by the caspase inhibitor Z-VAD-FMK.

Conclusion: These findings suggest that AT2R overexpression trigger INS-1 cells apoptosis and dysfunction of insulin secretion, which may be mediated via cell death signaling pathway that is dependent on activation of caspase family.



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PS 017 Transgenic animal models of type 1 and type 2 diabetes

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Expression of the chemokine receptors CXCR6 and CX₃CR1 on CD8⁺ T cells in diabetic RIP-LCMV mice

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Background and aims: In Type 1 Diabetes (T1D) self-destructing lymphocytes are attracted to the islets of Langerhans by proinflammatory chemokines. The IFN γ inducible transmembrane chemokines CXCL16 and CX₃CL1 with their receptors CXCR6 and CX₃CR1 and their sheddase ADAM 10 could be novel targets for T1D therapy. Here we investigated the expression of, and ADAM10 during the pathogenesis of T1D in a mouse model.

Materials and methods: We used the well-established RIP-LCMV model of T1D. As a target autoantigen in the β -cells, RIP-LCMV mice express the glycoprotein (GP) or the nucleoprotein (NP) of the lymphocytic choriomeningitis virus (LCMV) under control of the rat insulin promoter (RIP). RIP-LCMV-GP mice develop T1D within two to three weeks after infection with LCMV, RIP-LCMV-NP mice within one to six months after infection. In order to evaluate the influence of CXCL16/CXCR6 and CX₃CL1/CX₃CR1, RIP-LCMV mice were crossed to either CXCR6 or CX₃CR1-deficient mice.

Results: Analysis of pancreas lysates from RIP-LCMV-GP mice collected at different times after infection revealed an upregulation of CXCL16 and CX₃CL1 in a time dependent manner after infection with LCMV. There is a strong increase of CXCL16 and CX₃CL1 production during the acute phase of infection until day 14. Thereafter in the chronic phase of the autoimmune destruction of the beta-cells the chemokine production is decreased compared to the peak at day 14, but remains elevated throughout the observation time. Histologic studies of pancreata from RIP-LCMV-GP mice collected at the same times after LCMV infection revealed an enhanced CX₃CL1/CX₃CR1 expression. In particular, CX₃CR1 was predominantly found in infiltrates of CD4 and CD8 T cells. Stimulation of lymphocytes from different organs (spleen, pancreatic lymph nodes, and pancreas) with immunodominant LCMV-peptides followed by intracellular staining of IFN γ to determine autoantigen-specific T-cells revealed a time dependent expression of CX₃CR1 on autoantigen-specific CD8 T-cells. Importantly, CX₃CR1 was expressed in a significantly higher frequency in pancreatic lymph nodes and spleen on specific CD8 T-cells, but was equally expressed in the pancreas. These data indicate a selective disappearance of specific CX₃CR1⁺ T cells after encounter of their target autoantigens in the pancreas. The peak of CX₃CR1⁺ antigen-specific T-cells from pancreatic lymph nodes to pancreas is delayed for two days indicating a migration of autoaggressive T-cells into the pancreas. Preliminary, incidence studies of RIP-LCMV-GP/NP x CXCR6^{GFP/GFP} mice showed a significant reduction of T1D development in both models compared with RIP-LCMV.

Conclusion: In summary our data demonstrate that both CXCL16 and CX₃CL1 are expressed after LCMV infection and remain elevated in the pancreas during the autoimmune destruction of beta-cells. However further analyses suggest that both the CX₃CL1/CX₃CR1 and the CXCL16/CXCR6 axis might be important for islet infiltration by antigen-specific T cells and subsequently the development of T1D.

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Commensal bacteria-LPS translocation does not promote the breakdown of T cell tolerance and autoimmune diabetes

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Background and aims: Type 1 diabetes results as a failure of the mechanisms that maintain immune tolerance. Genetic and environmental factors contribute to render potentially autoreactive T cells into pathogenic effectors. Both, CD8⁺ and CD4⁺ T cells override tolerance and cooperate to progressively destroy the beta cells of the pancreas. Interestingly, it has recently been shown that commensal bacteria can shape and induce anti-self T cell responses. These observations suggest that bacterial products may promote the

breakdown of T cell tolerance. Here we have addressed whether the systemic translocation of LPS from commensal bacteria promotes the breakdown of peripheral T cell tolerance to pancreatic antigens.

Materials and methods: To address this question, we have utilized transgenic mice that express a well-characterized model antigen, influenza HA, in the beta cells of the pancreas. These mice are profoundly tolerant of the HA antigen in both the CD8⁺ and the CD4⁺ T cell compartments. Mild irradiation promotes the breakdown of T cell tolerance and the onset of autoimmune diabetes. One of the effects of irradiation is the systemic translocation of LPS from commensal bacteria. To assess whether bacterial LPS is responsible for the breakdown of tolerance observed we have treated mice with a cocktail of antibiotics in the drinking water in order to prevent translocation.

Results: Our results demonstrated that antibiotic treatment can efficiently prevent the systemic translocation of LPS induced by irradiation. Surprisingly, the absence of LPS translocation did not prevent the onset of autoimmune diabetes in transgenic mice. Beta cell-specific CD8⁺ T cells proliferated extensively in response to self-antigen cross-presentation in the draining lymph nodes of the pancreas, differentiated into effector cells and infiltrated the islets of Langerhans inducing disease in both antibiotic treated mice and controls. Analyses of the CD11c⁺ antigen presenting cells demonstrated that irradiation induces their activation as measured by the enhanced expression of CD80, CD86, CD70, CD40 and MHC II. However, antibiotic treatment did not completely reverse this activation in all the dendritic cell subsets analyzed.

Conclusion: Our results indicate that commensal bacteria LPS translocation is not sufficient to promote the breakdown of CD8⁺ T cell peripheral tolerance and the onset of autoimmune diabetes.

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Predictive value of decision-tree analysis of blood gene expression profiles for islet infiltration in the LEW.1AR1-iddm rat model of type 1 diabetes

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Background and aims: The LEW.1AR1-iddm rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range between 40 and 60 days after birth resulting in progressive beta cell destruction and overt diabetes. It was the aim of this study to generate a prediction model (decision-tree) for islet infiltration that based on gene expression profiles of peripheral blood mononuclear cells (PBMC) of prediabetic normoglycaemic LEW.1AR1-iddm rats.

Materials and methods: Normoglycaemic LEW.1AR1-iddm and LEW.1AR1 control rats were killed at the age of 40 - 60 days. Serial pancreatic sections were stained with Haematoxylin-Eosin (HE) to document the state of islet infiltration. RNA was isolated from purified PBMCs for PCR-Array analysis and RT-PCR. Gene expression data of proinflammatory cytokines, anti-inflammatory cytokines and T-cell markers were analyzed by WEKA data mining tool using C4.5 algorithm to generate a decision-tree to predict islet infiltration.

Results: At the stage of organ preparation all rats were normoglycaemic (blood glucose level 5 - 7.5 mmol/l) and were discriminated by the status of infiltration (uninfiltrated, infiltrated). The gene expression profile of PBMCs followed a two-peak model with high expression of chemokines at day 40, 60 and downregulation at day 50. The cytokine- and chemokine genes *Ccl2*, *Cxcl11*, *Ccl21*, *IL-18*, *TNF* (2.1-2.7fold), *Cxcl1*, *Cxcl2*, *Cxcl3* (3.6-5.4fold) and *Ccl7*, *Ccl24* (6.9-8.8fold) were upregulated at day 40 in rats with islet infiltration and facilitate recruitment of immune cells to the pancreas. A downregulation of cytokine- and chemokine genes was observed for *C4b*, *Ccl1*, *Ccl17*, *Ccl24*, *Ccr2*, *IL-1a*, *IL-1b*, *IL-6*, *IL-6r*, *IL-9*, *Tlr4*, *Tlr7* and *TNF* in combination with upregulated genes *Cxcl2*, *Cxcl3* and *IL-22* at day 50 in rats with progressive islet infiltration. Upregulation was also detected for *C4b*, *Ccl1*, *Ccl2*, *Ccl21*, *Ccl22*, *IL-18* (2.3-5.3fold) and was accompanied by strong expression of *Cxcl1* (33.8fold), *Cxcl3*, *Cxcl2* (10.2-11.7fold), *Ccl7* (49.7fold) and *Ccl24* (129.2fold) in infiltrated animals and highlight a significant inflammation state in the blood. Decision tree analysis showed specific combinations of genes with predictive character at different time points of islet infiltration. At the early stage of islet infiltration (d40) *IL-1 β* proved to be the most predictive marker of islet infiltration. At d45 *IL-1 β* and *TNF- α* were characteristic for islet infiltration while with progression of islet infiltration and beta cell destruction the regulatory genes *CTLA4* and *CD25* (d50), *NRP1* and *IL-1 β* (d55) and *IL-10* (d60) gained decisive power.

Conclusion: The process of islet infiltration is mirrored by a two peak model of proinflammatory and antiinflammatory chemokines/cytokines expression in PBMCs. A proinflammatory gene expression profile has predictive power for the early period of islet infiltration while anti-inflammatory/regulatory genes dominate the progressive period of islet infiltration. In the late period of islet infiltration before onset of manifest hyperglycaemia proinflammatory genes showed a second peak. Thus, in risk patients the gene expression profiles in PBMCs could be an attractive strategy to monitor the state of islet autoimmunity in combination with the titer of autoantibodies.

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A LADA subform in the LEW.1AR1-iddm rat, a model for human type 1 diabetes

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Background and aims: The LEW.1AR1-*iddm* rat as an animal model of human type 1 diabetes was characterized during diabetes development by an immune cell infiltrate of CD4 and CD8 T cells as well as CD68 macrophages with proinflammatory cytokine expression in pancreatic islets leading to apoptotic beta cell loss. In this rat model a late onset and slowly developing subform of diabetes could be distinguished from the early and quickly progressing one.

Materials and methods: Twice before and immediately after diabetes manifestation pancreatic tissue was analyzed, obtained from the rats by sequential biopsies and of the residual organ. The analyses were performed by immunohistochemistry and in situ RT-PCR for the immune cell composition and the cytokine pattern of the infiltrate including proliferation and apoptosis rate of the pancreatic beta cells in comparison with the normoglycaemic controls.

Results: The pancreatic islets from normoglycaemic LEW.1AR1-*iddm* rats showed no signs of immune cell infiltration and cytokine expression. Diabetes manifestation took place between day 55 and day 65 for the early developing subform. In this fulminant subform the immune cell infiltrate was equally composed of CD8 T cells and CD68 macrophages started 3 to 5 days before diabetes manifestation. Immediately after infiltrating the islets the immune cells expressed the proinflammatory cytokines, IL-1 β and TNF- α . Ultrastructurally confirmed apoptotic beta cell death showed an apoptosis rate between 1.7–3.7 % in the TUNEL assay whereas the proliferation rate of beta cells ranged from 0.7–2.5 %, as identified by double immunofluorescence staining of Ki67 and insulin. Diabetes manifestation occurring after day 80 showed a prolonged infiltration process of more than 10 days before disease onset. The immune cell infiltrate composition comprised 2/3 CD68 macrophages and only 1/3 CD8 T cells. These infiltrating islets strongly expressed the chemokine Ccl2 especially attracting macrophages into the islets in contrast to islets from the fulminant subform. The proinflammatory cytokine expression pattern showed a strong expression of IL-1 β before diabetes manifestation and later on after onset concomitant with the expression of TNF- α . The apoptosis rate was lower than in the fulminant subform with values between 0.8–2.0 % whereas the proliferation rate of beta cells ranged from 0.9–3.2 %.

Conclusion: Heterogeneities in the LEW.1AR1-*iddm* rat colony were observed during diabetes development. Animals with a later onset of diabetes showed a milder diabetes form with a high number of surviving beta cells. This subform of the LEW.1AR1-*iddm* rats starting later than day 80 with an infiltration dominated by macrophages and a slower beta cell loss resembles very well the LADA (Latent Autoimmune Diabetes in Adults) subform in human adults developing autoimmune diabetes at an age >35 years. The lower frequency of T cells and the lower expression level of TNF- α in the immune cell infiltrate opens the perspective to develop specific immunomodulatory treatments for the LADA type of autoimmune diabetes.

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Aberrant hypothalamic expression of the master clock gene Per1 at diabetes onset in non-obese diabetic mice

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Background and aims: Fundamental metabolic and immunological processes follow a circadian periodicity, which is under control of an endogenous rhythm generator in the hypothalamic suprachiasmatic nucleus (SCN). Previous studies demonstrated disturbed rhythmicity of energy metabolism and physical activity in newly diabetic non-obese diabetic (NOD) mice. We therefore hypothesized that the pathogenesis of insulin-deficient diabetes in NOD mice is associated with disturbed expression of the period circadian clock protein homolog 1 (Per1), a master regulator of circadian rhythmicity in the hypothalamus.

Materials and methods: Female C57BL6 mice and prediabetic (normoglycemic) NOD mice (70–80 days old) as well as newly diabetic (< 5 d) NOD mice were kept at a 12-h light / 12-h dark cycle. The physical activity (registered as “counts”) and the respiratory exchange rate (RER) of the animals were monitored in an automated modular system for comprehensive metabolic phenotyping. Immunohistochemistry of the SCN was performed to quantify the expression of the master clock gene Per1 and of the transcription factor cFos, which reflects general cerebral activity.

Results: C57BL6 mice as well as non-diabetic and newly diabetic NOD mice showed comparable low physical activity during light phases whereas during dark phases diabetic NOD mice showed a two-fold lower activity than C57BL6 and non-diabetic NOD mice ($p < 0.05$). The altered pattern of physical activity of diabetic NOD mice was associated with a constantly decreased RER in the light (0.82 ± 0.01) and dark phases (0.83 ± 0.01) when compared to non-diabetic NOD mice (RER light phase: 0.88 ± 0.01 ($p < 0.001$); RER dark phase: 0.95 ± 0.01 ($p < 0.001$)). In non-diabetic NOD mice, hypothalamic expression of Per1 was high (Per1 positive cell nuclei in SCN: 126 ± 19) at Zeitgeber (ZT) 14 (2 h after light-on). However, at ZT02 (2 h after light-off) the Per1 expression in these animals did not show the expected reduction that is observed in C57BL6 mice (26 ± 11) but remained elevated (108 ± 14) ($p < 0.01$). In NOD mice, the high levels and the lack of circadian rhythmicity of Per1 expression persisted after the onset of diabetes (ZT14: 121 ± 23 , ZT02: 97 ± 19). Higher Per1 expression in NOD mice was associated with a loss of the periodicity of their hypothalamic cFos expression after diabetes onset.

Conclusion: In conclusion, the finding of an aberrant hypothalamic expression of the master clock gene Per1 in prediabetic NOD mice points to a role of disturbed circadian rhythmicity during the development of insulin-deficient diabetes in this model of type 1 diabetes.

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Targeted deletion of SIRT6 in pancreatic beta cells leads to glucose intolerance

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Background and aims: Sirtuin 6 (SIRT6) has emerged as a key regulator of glucose and fatty acid metabolism in liver and adipose tissues. However, the role of SIRT6 in pancreatic beta-cells has not reported. In this study, we examined the physiological role of SIRT6 in pancreatic beta-cell function and systemic glucose homeostasis.

Materials and methods: A Cre-LoxP system was used to generate beta-cell-specific SIRT6 knockout (BS6KO) mice. Metabolic and histological analyses were carried out in BS6KO mice as well as littermate controls after normal chow diet (NCD) or high fat diet (HFD) for 16 weeks. Additionally, isolated islets from BS6KO mice were used to investigate the effect of SIRT6 deletion on insulin secretion and beta-cell viability.

Results: While their fasting glucose and body weight were comparable with wild-type mice on NCD, BS6KO mice fed the HFD induced more weight gain and exhibited hyperglycemia in both nonfasting and 16-h fasting conditions. Oral glucose tolerance test revealed that BS6KO mice had severe glucose intolerance, which was paralleled by a reduction in insulin-immunoreactive

pancreatic beta-cell mass. Mimicking this, islets from BS6KO mice exhibited profound defects in the glucose-stimulated insulin secretion. On the contrary, normal islets transduced with SIRT6 adenovirus were resistant against cytokine- and lipo-toxicity.

Conclusion: In addition to being essential for maintaining glucose homeostasis, SIRT6 is involved in beta-cell function by protecting beta-cells against exogenous insults and thereby maintaining insulin secreting capacity.

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Glycogen over-accumulation in beta cells affects insulin secretion and glucose homeostasis

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Background and aims: The presence of detectable amounts of glycogen in β -cells of diabetic patients has led to the notion that excessive glycogen accumulation is involved in glucotoxicity and β -cell dysfunction. However, the causal contribution of β -cell glycogen to the pathophysiology of diabetes remains elusive. The regulatory subunit Protein targeting to glycogen (PTG) induces the dephosphorylation of glycogen synthase, resulting in a hyperactivation of this enzyme and enhanced glycogen buildup. In this study, to directly investigate the effect of increased glycogen storage in β -cells on glucose homeostasis and β -cell function, we have generated a transgenic mouse model over-expressing PTG in these cells.

Materials and methods: Mice over-expressing PTG in β -cells (β PTG) were generated by crossing conditional PTG transgenic mice with RIPCre mice. RIPCre littermates were used as controls (Ctrl) in all experiments. Glucose homeostasis was measured by intraperitoneal glucose tolerance test (IP-GTT) and insulin sensitivity test (ITT). Pancreatic insulin positive area was measured by morphometry. Glycogen was detected by PAS staining and by enzymatic methods after precipitation in 66% ethanol and digestion with α -amylglucosidase. Insulin secretion was measured by static incubation of isolated islets and quantified by ELISA.

Results: β PTG mice were born at expected ratios and showed no differences in growth and body weight. PAS staining revealed heterogeneous glycogen accumulation in β -cells. β PTG mice exhibited a mild impairment in glucose tolerance (AUC β PTG vs Ctrl: 665 ± 65 vs 429 ± 56 , $p < 0.05$) and normal peripheral insulin sensitivity. Random plasma glucose levels were indistinguishable from controls but fasting glycaemia was significantly lower (β PTG vs Ctrl: 45 ± 1.3 mg/dl vs 55 ± 2 mg/dl, $p < 0.05$). Correlating with their mild glucose intolerance, morphometric analysis revealed a 33% reduction in pancreatic insulin positive area in adult β PTG mice relative to controls (β PTG vs Ctrl: $0.36 \pm 0.01\%$ vs $0.54 \pm 0.04\%$, $p < 0.01$). To address an additional effect of accumulated glycogen on insulin secretion, islets were isolated from β PTG and controls. Insulin content per islet was similar between β PTG and controls: 500 ± 49 ng vs 470 ± 35 ng, NS). Islets isolated from fed ad libitum β PTG mice contained 5-fold more glycogen than controls (β PTG vs Ctrl: 5.5 ± 0.4 ng/islet vs 1.1 ± 0.3 ng/islet, $p < 0.001$) but, after an overnight fasting, glycogen was depleted to undetectable levels in both groups. Strikingly, β PTG islets from fed mice exhibited enhanced glucose-induced insulin secretion (percentage of release relative to insulin content at 16.7 mM glucose, β PTG vs Ctrl: $1.2 \pm 0.15\%$ vs $0.68 \pm 0.11\%$, $p < 0.05$). This improvement was lost after fasting (β PTG vs Ctrl: $0.57 \pm 0.12\%$ vs $0.58 \pm 0.11\%$, NS), suggesting that increased glycogen levels in fed β PTG islets were the reason for the enhanced glucose-induced insulin secretion.

Conclusion: Our results support the idea that high glycogen levels may contribute to β -cell loss. Intriguingly, our findings also show that glycogen plays a beneficial role for glucose-induced insulin secretion. These contradictory effects of increased glycogen storage on β -cell physiology expose the possibility that the accumulation of glycogen has a time and/or dose dependent influence on these cells. Further investigations will be needed to define the precise mechanisms and progression of such events.

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Reduction in pancreatic beta cell mass caused by enhanced expression of Cdkn1c via interaction between C/EBP β and epigenetic control

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Background and aims: The relationship between pancreatic β -cell mass and the onset and progression of type 2 diabetes mellitus has received a lot of attention in recent years. Our research focused on the Cdkn1c gene, which codes for a cell cycle inhibitor, because it is involved in the regulation of pancreatic β -cell mass. The Cdkn1c gene is an imprinting gene whose expression is controlled by the non-coding RNA Kcnq1ot1. We found that when the expression of Kcnq1ot1 decreased, epigenetic alterations induced an increase in the expression of Cdkn1c, which, in turn, led to a decrease in the pancreatic β -cell mass. We have already reported that the transcription factor C/EBP β accumulates in the pancreatic β -cells of high fat-fed mice. It has been reported that a binding motif of C/EBP β is present in the Cdkn1c promoter. On the basis of these findings, we hypothesize that the accumulation of C/EBP β in pancreatic β -cells with reduced expression of Kcnq1ot1 causes further enhancement of Cdkn1c expression and promotes pancreatic β -cell failure.

Materials and methods: C/EBP β was overexpressed in MIN6 cells treated with an inhibitor of epigenetic modification. In addition, Kcnq1ot1-truncated C/EBP β -overexpressing mice (KT mice) were produced by cross-breeding Kcnq1ot1-truncated mice with pancreatic β cell-specific C/EBP β -overexpressing mice.

Results: The findings showed that the expression of Cdkn1c in MIN6 cells was not influenced by the expression level of C/EBP β , however, it was enhanced by treatment with an inhibitor of epigenetic modification, and was further enhanced by C/EBP β overexpression. Studies using chromatin immunoprecipitation (ChIP) assays have shown that the binding between C/EBP β and the Cdkn1c promoter was significantly enhanced during treatment with the inhibitor than under normal conditions. In vivo studies have shown that compared to C/EBP β -overexpressing mice and wild-type mice, KT mice show significantly higher blood glucose levels, smaller pancreatic β -cell mass, and enhanced expression of Cdkn1c in the pancreatic islets.

Conclusion: The findings suggest that the introduction of epigenetic modifications into MIN6 cells or pancreatic β -cells may lead to enhanced binding of C/EBP β to the Cdkn1c promoter, then an increase in the expression levels of Cdkn1c, and a decrease in pancreatic β -cell mass.

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Tbc1d1 knockout increases glucose-stimulated insulin secretion from isolated mouse pancreatic islets

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Background and aims: The GTPase-activating protein TBC1D1 is known to play a crucial role in the translocation process of GLUT4 storage vesicles in skeletal muscle. Previous studies showed the expression of TBC1D1 in the pancreas, while the physiological functions and the differential expression within the pancreas are unknown. In our project we investigate the role of TBC1D1 in mouse pancreatic islets of C57BL/6J mice with β -cell specific overexpression of *Tbc1d1* and with a global knockout for *Tbc1d1*.

Materials and methods: To investigate the role of TBC1D1 in isolated mouse pancreatic islets we measured changes in glucose-stimulated insulin secretion (GSIS), palmitate uptake as well as protein and gene expression. Statistics are calculated by two-tailed, unpaired student's t-test.

Results: In Western blots TBC1D1 was highly enriched in the pancreatic islets. It could not be detected in *Tbc1d1*-deficient islets. Isolated islets of *Tbc1d1*-deficient mice showed a significant increase in GSIS (1012.06 ± 137.65 % vs. 1593.36 ± 229.47 %, $n=7$, $p=0.03$). In contrast, isolated islets which overexpress *Tbc1d1* did not show any changes in insulin secretion (625.87 ± 147.68 % vs. 597.10 ± 164.93 %, $n=7$, $p=0.67$). In addition, ¹⁴C-palmitate uptake in isolated islets from *Tbc1d1*-deficient mice ($n=6$) and cul-

tivated MIN6 cells after *Tbc1d1* knockdown ($n=5$) was significantly increased compared to respective controls (0.9 ± 0.038 fmol \times min $^{-1}$ \times islet $^{-1}$ vs. 1.28 ± 0.069 fmol \times min $^{-1}$ \times islet $^{-1}$, $p<0.05$ and 0.994 ± 0.039 pmol \times min $^{-1}$ \times mg $^{-1}$ protein vs. 1.251 ± 0.057 pmol \times min $^{-1}$ \times mg $^{-1}$ protein, $p<0.01$, respectively). Gene expression analysis of *Tbc1d1* knockout islets revealed the significant up-regulation of *Tbc1d4* and fatty acid receptors *Ffar3*, *Gpr119* and *Gpr120* ($n=8$), genes responsible for cellular lipid sensing.

Conclusion: We showed the enriched expression of TBC1D1 in mouse pancreatic islets compared to the exocrine tissue. The significant increase in glucose-stimulated insulin secretion of *Tbc1d1*-deficient islets demonstrates a stimulatory effect of *Tbc1d1*-deficiency. The increased ^{14}C -palmitate uptake in *Tbc1d1*-deficient islets might indicate a function for TBC1D1 in the regulation of lipid metabolism, presumably by regulating expression, transport or activity of fatty acid receptors and transporters. In a physiological context we assume that *Tbc1d1*-deficiency beneficially compensates for insulin resistance and has a protective effect on lipid overload.

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PS 018 Control of insulin exocytosis

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Glucose-induced ATP generation in alpha and beta cells matches the control ranges for glucagon and insulin release

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Background and aims: ATP is a crucial signal in glucose-stimulated insulin secretion, coupling metabolism to depolarization by blocking ATP-sensitive K^+ channels. ATP has been attributed various roles also in models of glucose-regulated glucagon release, but consensus about mechanisms is still lacking. The aim of the present study was to clarify whether glucose-induced ATP generation in α -cells differs from that in β -cells in a manner consistent with a role in signal transduction of glucagon secretion. Since Ca^{2+} is a major trigger of islet hormone secretion we also determined the relationship between Ca^{2+} and ATP.

Materials and methods: Islets from transgenic GLU-RFP mice, expressing red fluorescent protein (RFP) in the α -cells, or from normal C57BL/6 mice were transduced with adenovirus expressing the ATP biosensor Perceval. Total internal reflection fluorescence (TIRF) microscopy was used to monitor the sub-plasma membrane ATP concentration ($[\text{ATP}]_{\text{pm}}$) in superficial islet cells. $[\text{Ca}^{2+}]_{\text{pm}}$ was recorded simultaneously using the indicators Fluo-4 or Fura Red. The identity of islet cells were determined by immunostaining and/or cell-characteristic $[\text{Ca}^{2+}]_{\text{pm}}$ responses.

Results: Elevating the glucose concentration from 3 to 20 mM induced β -cell-typical responses with slow $[\text{ATP}]_{\text{pm}}$ oscillations that were synchronized among RFP-negative cells in the islets from GLU-RFP mice. However, the RFP-positive α -cells showed erratic reactions indicative of molecular interference between Perceval and RFP. We therefore analyzed $[\text{ATP}]_{\text{pm}}$ in normal mouse islets, identifying α - and β -cells by cell-characteristic $[\text{Ca}^{2+}]_{\text{pm}}$ responses to low and high glucose as well as to glutamate. Glucose elevation from 1 to 5 mM, which inhibits glucagon release, induced small increases in average $[\text{ATP}]_{\text{pm}}$ in both α - ($6 \pm 2\%$ increase of Perceval fluorescence) and β -cells ($9 \pm 1\%$). Subsequent elevation to 20 mM glucose, which stimulates insulin secretion, resulted in a much less pronounced increase of $[\text{ATP}]_{\text{pm}}$ in α - than in β -cells ($12 \pm 3\%$ versus $49 \pm 2\%$ above the initial baseline). However, the α -cell response was left-shifted since $[\text{ATP}]_{\text{pm}}$ at 5 mM glucose was 45% of that at 20 mM as compared to 19% in the β -cells. At 3 mM glucose $[\text{ATP}]_{\text{pm}}$ was stable in β -cells but showed irregular oscillations in α -cells that were antiparallel to oscillations of $[\text{Ca}^{2+}]_{\text{pm}}$. At 20 mM both α - and β -cells showed $[\text{ATP}]_{\text{pm}}$ oscillations that were antiparallel to those of $[\text{Ca}^{2+}]_{\text{pm}}$.

Conclusion: $[\text{ATP}]_{\text{pm}}$ shows a left-shifted glucose dependence in α -cells as compared to β -cells, consistent with ATP involvement in glucose-inhibited glucagon release. $[\text{Ca}^{2+}]_{\text{pm}}$ oscillations in both cell types are antiparallel to those of $[\text{ATP}]_{\text{pm}}$ probably reflecting energy-requiring Ca^{2+} transport.

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Biphasic nature of glucose-induced insulin secretion is independent on energy metabolism

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Background and aims: Insulin secretion in vivo is biphasic but the underlying mechanisms remain enigmatic. Many intracellular processes have been reported to follow an oscillatory/biphasic pattern and have thereby been implicated as regulators of insulin release kinetics. In particular, the sustained second phase of insulin secretion has been suggested to be driven by beta-cell energy metabolism. However the efforts to dissect the relationship between the oxidative metabolism and insulin secretion have been hampered by the lack of reliable technology for analysing both processes in real-time.

Materials and methods: We resolved the two phases of insulin secretion in intact mouse islets using high-resolution 3-D multiphoton confocal imaging with polar tracers. Cytosolic ATP/ADP ratio (using a recombinant probe Perceval) or Ca^{2+} levels ($[\text{Ca}^{2+}]_{\text{p}}$, using trappable dyes) were recorded in parallel with secretion to correlate energy metabolism and intracellular signalling events to insulin granule exocytosis.

Results: Exocytosis in pancreatic beta-cells was triggered by the glucose-induced elevation of cytosolic ATP and Ca^{2+} influx into the cytosol. Insulin secretion correlated strongly with changes in $[\text{Ca}^{2+}]_{\text{p}}$, but showed little correlation with bi-phasic kinetics of ATP increases. Other substrates of oxida-

tive metabolism affecting cytosolic ATP/ADP levels, such as amino acids or methyl succinate, had no effect on the second phase of secretion. At the same time, changes in $[Ca^{2+}]_i$ show a strong temporal correlation with exocytosis. The effects of $[Ca^{2+}]_i$ on insulin exocytosis were potentiated by agents producing an elevation of intracellular cAMP (like GLP-1).

Conclusion: These findings illustrate the power of on-line high-resolution monitoring of multiple parameters in living systems. Our data suggest a hierarchy of intracellular signalling events in the control of insulin secretion. We propose that insulin release kinetics is principally determined by variations of signalling events (including $[Ca^{2+}]_i$) rather than energy metabolism. The latter is likely to play the triggering role but the temporal fine-tuning is exerted via more distal events.

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Identification of Rab27a-GAP-interacting proteins and its functional analysis in pancreatic beta cells

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Background and aims: Membrane trafficking is crucial for the regulation of the size of the readily releasable pool of insulin granules and for the recovery of insulin secretory membranes. Rab27a, which is a member of the Rab family, have been considered to control of pre-exocytosis in pancreatic-beta cells through its GTP-dependent effectors. In contrast, the GDP-bound form has been regarded as an inactive form, because the functional binding partner of this form has not been identified. We previously identified coronin 3 as a novel GDP-dependent effector of Rab27a in pancreatic beta-cells. Moreover, we found that the insulin secretagogue glucose caused a shift from the GTP- to GDP-bound Rab27a via Rab27a-GAP, resulting in the regulation of endocytosis of insulin secretory membranes. Taken together, GTP- and GDP-bound Rab27a regulates insulin secretion at the pre-exocytotic and the endocytotic stages, respectively. In the present study, we investigated the underlying mechanisms by which Rab27a-GAP controls endocytosis of insulin secretory membranes in pancreatic beta-cells.

Materials and methods: Affinity column chromatography was performed to identify Rab27a-GAP interacting proteins. Extracts from the insulin-secreting beta-cell line, MIN6, were loaded onto glutathione-sepharose 4B beads coated with GST-Rab27a-GAP. The proteins bound to the columns were eluted and were analyzed by peptide mass fingerprinting. For binding assay, immunoprecipitation analysis was performed using COS7-cells expressing Rab27a-GAP. For the observation of the intracellular localization of Rab27a-GAP and Arf6-GEF, MIN6-cells were analyzed with a TIRF microscopy system or a confocal laser microscopy system.

Results: We identified Arf6-GEF as a Rab27a-GAP interacting protein. Arf6-GEF is a guanine nucleotide exchange factor that converts Arf6 from the GDP- to the GTP-bound form, resulting in the regulation of endocytosis through clathrin-coated vesicle formation. We found that Rab27a-GAP directly interacted with Arf6-GEF. The C-terminal region of Rab27a-GAP bound the PH domain of Arf6-GEF. Glucose stimulation induced the redistribution of both Rab27a-GAP and Arf6-GEF to the vicinity of the plasma membrane. Silencing of Arf6-GEF inhibited the glucose-induced redistribution of Rab27a-GAP. In contrast, the redistribution of Rab27a-GAP did not occur in Arf6-GEF-silencing cells.

Conclusion: These results indicate that Arf6-GEF is required for the glucose-induced redistribution of Rab27a-GAP. We previously reported that GDP-bound Rab27a regulates the retrograde transport of the internalized secretory membrane, the stage after the GTP-bound Arf6-dependent formation of clathrin-coated vesicles. We therefore propose a model where Rab27a-GAP is involved in both Rab27a and Arf6 signaling and plays a pivotal role in membrane trafficking for insulin secretion.

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Temperature-dependent effects of high glucose and potassium depolarisation on insulin granule mobility and exocytosis in insulin-secreting MIN6 cells

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Background and aims: It is widely assumed that the first phase of glucose-induced insulin secretion can be mimicked by K⁺ depolarization. We found, however, that the insulinotropic efficacy was differently affected by lowering the temperature. We thus compared parameters of insulin granule mobility and secretion during glucose stimulation and K⁺ depolarization at different temperatures.

Materials and methods: Insulin granules were labeled by transient transfection with insulin-EGFP and the granules present in the submembrane space were imaged by TIRF microscopy. Single MIN6 cells were continuously perfused with oxygenated HEPES-buffered Krebs-Ringer medium containing the respective stimuli at 37 °C, 32 °C, and 22 °C. The image files (1 sequence = 200 images) were evaluated by an in-house written program. The insulin secretion of MIN6 pseudo-islets was measured by perfusion with ELISA of the fractionated efflux.

Results: At 37 °C stimulation of the perfused MIN6 pseudo-islets with 30 mM glucose and, after a wash-out period of 10 minutes, 40 mM K⁺ increased insulin secretion. K⁺ was about twofold more effective than glucose. At 32 °C, K⁺ was even more effective than at 37 °C, and was markedly but only transiently effective at 22 °C. Relative to K⁺, glucose lost efficacy at 32 °C and was virtually ineffective at 22 °C. When the stimuli were applied in the reversed sequence K⁺ was of unchanged efficacy, but glucose was almost ineffective at all temperatures. The same experimental protocol was used to measure the mobility of insulin granules in the submembrane space of perfused single MIN6 cells. Under control conditions (3 mM glucose, 37 °C), the mean number of submembrane granules was 357 ± 37 per cell footprint at the beginning of image acquisition. 33.5 ± 1.4 % of these remained visible throughout the sequence (long-term residents). Newly arrived granules made up 8.9 ± 0.6 % per image, equivalent to about 32 granules. The number of departing granules closely mirrored that of arriving granules (mobility in the z-dimension). The short-term residents (present for ≤ 1 s) amounted to 82.2 ± 0.9 % of the total number of granules (7547 ± 928) in a sequence. At all temperatures stimulation with glucose and K⁺ led to an increase in the number of arriving and departing granules, independent of the sequence of exposure. Consequently, the total number of granules per sequence was increased during phases of stimulation. The caging diameter (mobility in the x/y-dimension) in contrast, decreased during glucose- and K⁺-stimulation at 37 °C, but not at 32 °C and 22 °C. The number of the long-term resident granules was similar for all three temperatures at basal glucose and when 40 mM K⁺ was applied first. However, when 30 mM glucose was applied first the number remained stable at 37 °C in contrast to a steady decline at 32 °C and 22 °C.

Conclusion: At all temperatures, glucose and K⁺ depolarization enhance the granule turnover in the submembrane space. This characteristic fits to K⁺-induced secretion, whereas the temperature-dependency of the caging diameter and of the long-term resident granules reflects the secretion pattern of the glucose stimulus when it is applied first. Additionally, glucose-induced secretion is associated with limited lateral mobility and/or longer presence of the granules at the plasma membrane.

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CaMKII-mediated metabolic memory of pancreatic beta cells controls insulin secretion and is inhibited by palmitate

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Background and aims: Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) functions both in regulation of insulin secretion and neurotransmitter release through common downstream mediators. Memory is the ability to acquire, to store and to evoke any kind of information. In CNS, the process behind this phenomenon in the Long-Term Potentiation (LTP) and is known

that it requires Ca^{2+} to occur. In addition, CaMKII is necessary to store information during LTP. In pancreatic β -cells, CaMKII plays pivotal role during GSIS process. Palmitate is a Saturated-fatty-acid-induced important for beta cell metabolism, especially during DM2 onset. It is well known that palmitate inhibits glucose-induced CaMKII phosphorylation and potentiates GSIS. Therefore, we investigate that pancreatic β -cells acquire and store the information as a form of “metabolic memory”, just as neurons store cognitive information, and, that palmitate inhibits this process.

Materials and methods: To test this hypothesis, we developed a novel paradigm of pulsed exposure of mice and human β -cells to intervals of high glucose, followed by a 24-hour consolidation period to eliminate any acute metabolic effects. After this period, we analyzed insulin secretion (by RIA), protein expression (by Western blot), response to a glucose-ramp and the glucose-induced Ca^{2+} influx.

Results: Strikingly, β -cells exposed to this high-glucose pulse paradigm exhibited significantly stronger insulin secretion. This metabolic memory was entirely dependent on CaMKII and was inhibited by Palmitate. We also observed, in pulse group, an increase in Ca^{2+} influx induced by glucose. In addition, metabolic memory was reflected on the protein level by increased expression of proteins involved in GSIS and Ca^{2+} -dependent vesicle secretion, such as GSK, Cav1.2, SNAP25, pCaMKII and pSynapsin. Finally, we observed in human islet elevated levels of the key β cell transcription factor MAFA.

Conclusion: In summary, like neurons, human and mouse β -cells are able to acquire and retrieve information in a process dependent on CaMKII that is inhibited by Palmitate.

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Islet microcapillary endothelial cells secrete an attenuating factor of insulin secretion

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Background and aims: Paracrine interactions and chemotactic signals exchanged within islet of Langerhans' endothelium and the endocrine cells have been proven critical for the development and organization of functional islet units. Moreover, autocrine and paracrine signals within the microenvironment of the islets can also modulate β -cell glucose-stimulated insulin secretion (GSIS). For instance, it has been shown that several extra-cellular matrix components, derived from islets' microcapillary endothelium (IME), regulate β -cell spreading and amplify insulin secretion. We aimed at investigating whether IME secrete other soluble factors that affect β -cell function and viability.

Materials and methods: Conventional conditioned-medium (CM) from IME (the MS-1 cell line and primary cultures of endothelial cells from rat islets) were prepared to study contact-independent IME- β -cell interactions. Standard GSIS assay in the absence or presence of insulin secretagogues was employed. In addition, cell viability assays (cell count, MTT assay and annexin/PI flow cytometry) and size-based fractionation of the CM were performed.

Results: Our data show that IME-derived CM contained soluble factor(s) that markedly attenuated GSIS from both INS-1E β -cell line and freshly isolated rat islets. Fractionation of the CM indicated that the active factor was a high molecular weight protein. This factor reduced GSIS without changing the rate of insulin biosynthesis, cell viability and cellular ATP content. Using the insulin secretagogues IBMX, KCl, tolbutamide and L-arginine, we found that only the second phase of insulin secretion was markedly reduced by the CM. This was accompanied with a significant decrease in cellular cAMP level following glucose stimulation. Furthermore, mass spectrometric analysis of several high molecular weight proteins in the active CM fraction are currently being analysed to identify the active attenuating factors of GSIS.

Conclusion: These findings support the hypothesis that a paracrine interaction between IME and β -cells modulates glucose-dependent insulin release and may contribute to maintaining glucose homeostasis.

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Glucose-stimulated insulin secretion is reduced after treatment with rosuvastatin calcium

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Background and aims: Statins are a family of drugs widely used to reduce the risk of cardiovascular disease by lowering the cholesterol levels. Rosuvastatin calcium (RS) is one of these highly prescribed statins and there are studies suggesting increased risk of diabetes for subjects on RS therapy. Whether RS influences insulin secretion from the beta-cell is yet to be established, but our group has previously shown that insulin secretion is highly sensitive to changes in plasma membrane cholesterol. Here, we aim to investigate the effects of RS on insulin secretion, exocytosis and Ca^{2+} influx through voltage-dependent Ca^{2+} channels in pancreatic beta-cells.

Materials and methods: The study was performed in INS-1 832/13 cells. These cells were treated with different concentrations of RS (20 nM, 200 nM, 2 μM and 20 μM) for various time spans (24/48 hour (h) and 1/2 month). Insulin secretion at 2.8 and 16.7 mM glucose (G) was measured by radioimmunoassay. Exocytosis was detected as changes in membrane capacitance evoked by a train of 10 depolarizations from -70 mV to 0 mV using whole-cell patch-clamp configuration.

Results: First we studied influence on glucose-stimulated insulin secretion (GSIS) in INS-1 832/13 cells treated with above mentioned concentrations of RS for 48 h. In cells treated with 200 nM, 2 μM and 20 μM RS insulin secretion at 16.7 mM G was significantly reduced (26%, 35% and 29% reduction; $p < 0.05$; $p < 0.01$ and $p < 0.001$ vs control, respectively; $n = 3-4$). Moreover, in cells treated with 2 μM and 20 μM RS insulin secretion at 2.8 mM G increased significantly (67% and 165% increase; $p < 0.05$ and $p < 0.01$ respectively) and there was a significant reduction in total protein content (25% and 50% reduction, $p < 0.05$ and $p < 0.001$ respectively) compared to control. The lowest concentration of RS tested had no effect on insulin secretion at 2.8 and 16.7 mM G. We then studied exocytosis in INS-1 832/13 cells treated for 24-48 h with the RS concentrations mentioned above. There was no significant difference in exocytosis in cells treated with the lower concentrations of RS as compared to control. However, in cells treated with 20 μM RS exocytosis and voltage-dependent Ca^{2+} current evoked by the first depolarization were significantly reduced (34% and 41% reduction, $p < 0.05$ and $p < 0.05$ vs control, respectively; $n = 29-30$). Hence, it is tempting to speculate that RS at 20 μM reduces exocytosis by altering properties of the voltage-dependent Ca^{2+} channels. In order to examine long term effects INS-1 832/13 cells were treated with 20 nM RS for 1 and 2 month. Subsequently, insulin secretion assay was performed but no significant change in insulin secretion was observed compared to control at this low concentration.

Conclusion: We conclude that RS, dose dependently reduces GSIS in INS-1 832/13 cells within 48 h. Thus, our data suggest that RS treatment might impair insulin secretion. Future experiments in *in vivo* and primary cells will further clarify if this indeed is the case.

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Improvement of glucose-stimulated insulin secretion by heparan sulfate proteoglycan syndecan-4 overexpression in mouse pancreatic beta cell line

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Background and aims: Heparan sulfate proteoglycans (HSPGs) are composed of a core protein to which extracellular glycosaminoglycan chains are attached. Syndecan-4 (Sdc4), one of the major heparan sulfate (HS)-containing core protein, is distributed on the cell surface, where it interacts with various proteins, including growth factors, morphogens and extracellular matrix components to regulate their signaling. We recently found that HS is localized exclusively around beta-cells in the islets of adult mice and is required for islet morphogenesis, beta-cell proliferation and insulin secretion. Furthermore, we found that the 3-O-sulfate groups of HS and Sdc4 are necessary for maintaining normal glucose-stimulated insulin secretion (GSIS), confirmed these results with RNA interference experiments. In this study, we attempted

to overexpress the core protein Sdc4 and to clarify the contribution of Sdc4 to beta-cell function.

Materials and methods: Subcloning of MIN6 cells, mouse pancreatic beta-cell line, was performed by the limiting dilution method. The cells were then screened and selected by an index of GSIS, KCl-induced insulin secretion, HS expression and mRNA expression of Sdc4. A stable transformant expressing the mouse Sdc4 was established by transfecting pCMV-mSDC4 and selected with hygromycin B. The stable transformant expressing the highest level of Sdc4 mRNA (by quantitative RT-PCR analysis) was used for further experimentation. GSIS activity was measured with a basal (2.8 mM) or stimulatory (11.2 or 25 mM) glucose concentrations.

Results: MIN6 cells were subcloned, and 30 clones were obtained. Two sublines were selected for this study, designated T3 and T16. T3 cells exhibit GSIS in a concentration-dependent manner, whereas T16 cells respond poorly to glucose. In the presence of KCl, T16 cells secreted almost the same amount of insulin as T3. Next, the expression of HS in these subclones was analyzed by western blot analysis. Immunoblot with anti-HS antibody showed a single band of 35 kDa, consistent with the reported size of the Sdc4, was detected in T3 cells. On the other hand, HS was not detected in T16 cells. In addition, the mRNA of Sdc4 was detected in T3 cells by RT-PCR analysis, while not detected in T16 cells. In Sdc4 overexpressed T16 (T16/mSdc4) cells, insulin secretory response exhibited 3.1-fold ($p<0.0001$) as glucose was increased from 2.8 to 25 mM; while, non-transfected (control) T16 cells exhibited 1.3-fold. Moreover, in Sdc4 overexpressed T3 (T3/mSdc4) cells, insulin secretory response exhibited 22.1-fold ($p<0.0001$) as glucose was increased from 2.8 to 11.2 mM; while, control T3 cells exhibited 2.7-fold ($p<0.005$).

Conclusion: Our data indicate that HSPG Sdc4 plays important role(s) in the glucose-stimulated insulin secretory response. However, there exists the difference in glucose responsiveness between T16/mSdc4 and T3/mSdc4 cells compared with control cells. These results indicate that it is necessary to consider the effect of HS chains covalently attached to the Sdc4 in addition to the overexpressed Sdc4.

PS 019 Cytokine-induced beta cell death

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A combined „omics“ approach identifies NMI as a novel cytokine-induced regulator of IRE1 α and JNK in pancreatic beta cells

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease with a strong inflammatory component. The cytokines interleukin-1 β (IL-1) and interferon- γ (IFN) induce endoplasmic reticulum stress and the unfolded protein response (UPR) and contribute to beta cell apoptosis in T1D. IRE1 α , one of the UPR mediators, triggers insulin degradation and inflammation in beta cells and is critical for the transition from “physiological” to “pathological” UPR. The mechanisms regulating cytokine-induced IRE1 α activation in beta cells remain to be clarified. To identify novel cytokine-induced regulators of IRE1 α we presently combined MAPPIT (MAMmalian Protein-Protein Interaction Trap)-based IRE1 α interactome with functional genomics analysis of beta cells exposed to IL-1 + IFN.

Materials and methods: By using ArrayMAPPIT, which identifies proteins that bind to the cytoplasmic domain of IRE1 α , we identified 31 putative IRE1 α -interacting proteins. These results were compared against our microarray and RNA sequencing data from INS-1E cells, FACS-purified rat beta cells and human islet cells exposed to IL-1 + IFN to identify IRE1 α -interacting proteins whose expression is modified by cytokines. Among the top 5 candidates, N-myc interactor (NMI) was chosen as the most promising cytokine-regulated IRE1 α -interacting protein. RNA interference was used to knockdown (KD) NMI in rat pancreatic beta cells and human islets and histological studies were done in pancreatic sections from non-obese diabetic (NOD) mice.

Results: Binary MAPPIT and immunoprecipitation confirmed NMI-IRE1 α interaction in rodent beta cells. NMI interacted with IRE1 α WT as well as with the kinase-defective K599A mutant IRE1 α , indicating that NMI-IRE1 α interaction is independent of IRE1 α activation. An increased expression of NMI was detected in islets from NOD mice with insulinitis, and in rodent or human beta cells exposed in vitro to IL-1 + IFN (between 3–10 fold increase; $p<0.05$ and $p<0.01$; $n=3-5$). NMI KD increased by 50% JNK phosphorylation ($p<0.001$; $n=4$), and increased cytokine-induced apoptosis by >10% ($p<0.05$; $n=4-7$) in rodent and human pancreatic beta cells after 24–48 h. Mechanistic studies demonstrated that NMI negatively modulates IRE1 α -dependent activation of JNK via the IRE1 α / TRAF2/ MKK7 pathway. Indeed, double KD of NMI with IRE1 α , TRAF2 or MKK7 significantly ($p<0.05$ - $p<0.001$; $n=4$) reverted the effects of NMI KD on JNK activation and/or apoptosis.

Conclusion: By using a combined omics approach, we identified NMI induction as a novel negative feedback mechanism that decreases IRE1 α -dependent activation of JNK and apoptosis in cytokine-exposed beta cells.

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LATS2 controls beta cell apoptosis by regulating the MOB1-Praja2 axis

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Background and aims: Apoptosis is the hallmark of loss of insulin-producing β -cells in both type of diabetes. We have recently identified Mammalian Sterile 20-like kinase 1 (MST1) as a key regulator of β -cell death and dysfunction in diabetes. On search for endogenous down-stream molecule(s) that mediate MST1 activation, we detected expression of the Large tumor suppressor 2 (LATS2) in β -cells whose activation was found under diabetic conditions. LATS2 is a serine threonine kinase and direct down-stream substrate of MST1, which plays an important role in regulating cell proliferation and apoptosis. So far, the role of LATS2 in the β -cell; whether LATS2 is activated

in diabetes and whether such activation triggers β -cell death, are not known and is investigated in this study.

Materials and methods: Isolated human islets and the rat β -cell line INS1 were exposed to a diabetic milieu (IL-1 β /IFN γ or increased glucose concentration (22.2 mM)). Bcl-xL, MOB1 (LATS2-associated protein), Ring-E3 ligase Praja2 (MOB1 negative regulator) and β -cell apoptosis (Caspase 3 & PARP cleavage) were analyzed by Western blotting. Praja2 and Bcl-xL expression were also analyzed by real-time PCR. LATS2 was overexpressed by plasmid transfection, LATS2 inactivation was performed by overexpressing dominant-negative LATS2 (dn-LATS2) or specific siRNA to LATS2. Bcl-xL turnover was performed in HEK 293 cells overexpressing Bcl-xL and LATS2. **Results:** LATS2 was activated in human islets and INS1 cells exposed to IL-1 β /IFN γ or increased glucose concentrations. This correlated with MOB1 up-regulation, decreased Praja2 expression and increased β -cell apoptosis. Overexpression of LATS2 itself increased MOB1 levels and β -cell apoptosis in INS1 cells and human islets indicating that LATS2 alone is sufficient to promote β -cell death. MOB1 interacts with and activates LATS2 kinase activity. Interestingly, MOB1 co-precipitation with LATS2 was increased in the presence the diabetic milieu compared to untreated INS1 cells suggesting that MOB1 is essential for the higher LATS2 activity under diabetic conditions. LATS2 overexpression in INS1 cells as well as in Bcl-xL-overexpressing HEK293 cells decreased mitochondrial anti-apoptotic protein Bcl-xL without changes in Bcl-xL mRNA levels; this suggested that the decrease in Bcl-xL expression occurred at a post transcriptional level and mediated the pro-apoptotic function of LATS2. Reciprocally, inhibition of endogenous LATS2 activity by siRNA knockdown or overexpression of dominant negative LATS2 protects β -cells from gluco- and cytokine-induced apoptosis demonstrated by decreased caspase-3- and PARP-cleavage as well as increased Bcl-xL levels. LATS2 knockdown down-regulated MOB1 and restored Praja2 levels under diabetic conditions in INS1 cells indicating a major role of MOB1 and Praja2 in the mechanism of stress-induced β -cell apoptosis. Our results indicate that LATS2, MOB1 and Praja2 regulate each other and may be a component of the previously uncharacterized stress-sensitive apoptotic pathway. Under normal conditions, Praja2 promoted cell survival by degrading MOB1 and then inhibiting LATS2 action, but prolonged diabetogenic stress decreased Praja2 expression, which allowed higher MOB1 levels and pro-apoptotic LATS2 signaling.

Conclusion: Our results show that LATS2 plays an important role in β -cell apoptosis and its inhibition may provide a new strategy to restore β -cell survival in diabetes.

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Identification of the ubiquitin ligase SCF(FBW7) as a novel regulator of the NF- κ B pathway in pancreatic beta cells

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Background and aims: Activation of the transcription factor NF- κ B by pro-inflammatory cytokines contributes to β -cell demise in animal models of type 1 diabetes (T1D). Thus, understanding on how NF- κ B is activated, is crucial to prevent β -cell death. SCF(FBW7) is an ubiquitin ligase with three isoforms, localised in the nucleolus, nucleus, and cytosol. It has been previously reported that FBW7 positively contributes to NF- κ B activity in cancerous cells. The aim of this study was to evaluate whether FBW7 modulates NF- κ B activation and survival of pancreatic β -cells.

Materials and methods: Specific siRNAs were used to downregulate FBW7 and β TrCP expression. Overexpression of FBW7 was carried out with plasmid encoding FBW7. Cell viability was assessed by the DNA-binding dyes Propidium Iodide and Hoechst 33342. NF- κ B activity was measured using a luciferase reporter assay. mRNA expression of NF- κ B-dependent genes, FBW7 and β TrCP was quantified by qPCR, while the proteins regulating NF- κ B activation were analysed by Western blot.

Results: IL-1 β +INF- γ decreased FBW7 mRNA expression in primary rat β -cells by 45%. Knockdown of FBW7 by siRNA (FBW7) significantly increased IL-1 β +INF- γ -mediated NF- κ B activation in INS-1E cells, primary beta cells as well as in human β -cell line as measured by a luciferase reporter promoter. This was accompanied by increased expression of the NF- κ B target genes Rantes and CCL19. There was also a two-fold induction of nitric oxide production ($p < 0.001$) in INS-1E cells. Conversely, overexpression of FBW7 significantly decreased cytokine-induced NF- κ B activation. The effects of FBW7 on NF- κ B activation were independent of I κ B- α degradation,

as it knockdown did not modify the pattern of IL-1 β +INF- γ -mediated I κ B- α degradation. On another hand, knockdown of FBW7 lead to accumulation of NF κ B2 precursor p100 and increased levels of its cleaved form p52 in the nuclear fraction of INS-1E cells after 4h. Induction of NF- κ B activity was reverted by a double knockdown approach of FBW7 and β TrCP, an E3 ligase, which was shown to be responsible for the proteosomal cleavage of p100 to p52. In line with these data, FBW7 knockdown potentiated the pro-apoptotic effects of IL-1 β +INF- γ in INS-1E cells (2-fold increase vs control siRNA, $p < 0.001$), FACS purified rat β -cells (1.9-fold increase vs control siRNA, $p < 0.001$) and human β -cell line.

Conclusion: These observations suggest that the E3 ligase FBW7 possesses an anti-apoptotic function in β -cells through regulation of the non-canonical NF- κ B pathway. Moreover, it unveils the involvement of this pathway in IL-1 β +INF- γ -induced NF- κ B activation.

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Fractalkine (CX3CL1), a new factor protecting beta cells against TNF- α

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Background and aims: We have previously shown the existence of a muscle-pancreas intercommunication axis in which fractalkine (CX3CL1), a CX3C chemokine produced by skeletal muscle cells, could be implicated. It has recently been shown that the fractalkine system modulates murine beta-cell function, and CX3CL1 expression is known to be regulated by TNF α , a cytokine that impacts negatively on beta cell function. However, the impact of CX3CL1 on islets and sorted beta-cells exposed to TNF α remains to be explored.

Materials and methods: Human islets and sorted beta and non-beta cells were used to measure CX3CL1 and CX3CR1 mRNA expression by RNAseq. Glucose induced insulin secretion (GIS: 1h; 16.7 mmol/l glucose) was measured using human islets following exposure to CX3CL1 (1–50 ng/ml) for 24h or rat beta cells exposed to 10 and 25ng/ml CX3CL1 for 48h alone or with 20 ng/ml TNF α for the last 24h. Rat beta-cell apoptosis (TUNEL) and proliferation (BrdU incorporation) were measured after 24h exposure to CX3CL1 (1 to 50 ng/ml) alone, or to CX3CL1 (20 ng/ml) for 48h with addition of cytomix (20 ng/ml TNF α , IL-1 β , INF γ) for the last 24h. Insulin signaling was analyzed by western blot in sorted rat beta-cells exposed to CX3CL1 (25ng/ml) for 48h with TNF α (20ng/ml) added for the last 24h. Data are mean \pm SE (5 independent experiments).

Results: CX3CL1 and CX3CR1 are both expressed in human islets: CX3CL1 is principally expressed by non-beta cells while its receptor is more expressed in beta-cells, as confirmed by immunofluorescence. Treatment with CX3CL1 for 24h had no significant impact on human islet and rat sorted beta cell GIS or insulin content, or on rat beta-cell proliferation and apoptosis. However, in rat sorted beta cells CX3CL1 completely blocked the adverse effect of TNF α on GIS (control: 2.64 \pm 0.22% content/h; TNF α : 1.46 \pm 0.11%; TNF α + 10 ng/ml CX3CL1: 3.19 \pm 0.12%; TNF α + 25 ng/ml CX3CL1: 2.62 \pm 0.31%; * $p < 0.05$ vs. control; ** $p < 0.05$ vs. TNF α alone) as well as partially protecting against the effects of cytomix on apoptosis (control: 0.14 \pm 0.06% TUNEL-positive beta-cells; cytomix: 0.59 \pm 0.22%; cytomix + 25 ng/ml CX3CL1: 0.26 \pm 0.08%; * $p < 0.05$ vs. control; * $p < 0.05$ vs. cytomix alone). Furthermore, CX3CL1 (25ng/ml) treatment of sorted rat beta-cells prevented the reduction in IRS2 protein levels and in glucose-induced phosphorylation of Akt and AS160 normally evoked by TNF α . Such treatment with CX3CL1 also prevented TNF α -induced phosphorylation of mTOR and NF κ B, without any impact on TNF α -induced basal ERK-1/2 phosphorylation. Finally, mTOR protein expression was reduced in cells pretreated with CX3CL1 and exposed to TNF α .

Conclusion: We demonstrate for the first time that CX3CL1 protects beta-cells from the adverse effects of TNF α on their function, likely by restoring phosphorylation of key proteins in the insulin signalling pathway. We further demonstrate that CX3CL1 protects rat beta-cells from cytomix-induced apoptosis. Our data suggest that CX3CL1 might be an important target to modulate the negative effects on beta-cells induced by inflammation in Diabetes

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IL-13 improves beta cell survival and protects against IL-1beta-induced beta cell death

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Background and aims: IL-13 is a cytokine classically produced by anti-inflammatory T-helper-2 lymphocytes. Interestingly, IL-13 is decreased in the circulation of type 2 diabetic patients and has recently been shown to impact positively on liver and skeletal muscle. Although IL-13 can impact positively on transformed beta-cell lines, its impact and mode of action on primary human or rodent beta-cell function and survival remain to be explored.

Materials and methods: Human and rat dispersed islet cells and sorted beta-cells were maintained in culture for 48 h in the presence of IL-13 (1 to 50 ng/ml) alone or in combination with IL-1 β (20 ng/ml). Insulin secretion in response to glucose (1h; 16.7 mmol/l) was measured by radioimmunoassay, proliferation by incorporation of BrdU over 48 h, cell death by TUNEL and protein expression/phosphorylation by western blot. Results are presented as mean \pm SE, n=3-5 independent experiments with statistical significance of differences assessed by Student's t test or ANOVA with Bonferroni post hoc test. **Results:** IL-13 did not affect human or rat beta-cell basal or glucose-stimulated insulin secretion, or rat beta-cell proliferation. IL-13 (10 ng/ml) decreased basal cell death in human (IL-13: 0.6 \pm 0.2% TUNEL⁺ cells vs. control: 1.1 \pm 0.3%; p<0.05) and rat beta-cells (IL-13: 0.02 \pm 0.01% TUNEL⁺ cells vs. control: 0.12 \pm 0.03%; p<0.05) and protected sorted rat beta-cells against IL-1 β induced apoptosis (normalized to control: IL-1 β : 2.6 \pm 0.4, p<0.05 vs. control; IL-13 + IL-1 β : 1.16 \pm 0.3%, p<0.05 vs. IL-1 β). However, IL-13 was unable to protect beta-cells from IL-1 β impaired glucose stimulated insulin secretion. IL-13 (10 ng/ml) induced phosphorylation of Akt in rat primary beta-cells after 60 min (99 \pm 41% increase; p<0.05) and increased IRS2 protein expression after 48 h (68 \pm 18% increase; p<0.05) without changing protein levels of Akt isoforms.

Conclusion: We show for the first time that IL-13 improves survival of primary beta-cells and protects against IL-1 β induced beta-cell death, without affecting proliferation or insulin secretion. These effects may be mediated through the IRS2/Akt signalling cascade. In addition to any beneficial effects on insulin target tissues, these data suggest that IL-13 may be useful for treatment of type 2 diabetes by preserving beta-cell mass or slowing its rate of decline.

Supported by: JDRF

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The role of sphingosine-1-phosphate-lyase in insulin-secreting cells

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Background and aims: Type 1 diabetes is an autoimmune disease with a strong inflammatory component. The sphingolipid metabolism and sphingosine-1-phosphate (S1P) play an important regulatory role in the development of several inflammatory disorders. S1P-lyase (SPL) cleaves irreversibly S1P in the last step of the sphingolipid catabolism. S1P was associated with protection against cytokine-mediated toxicity in insulin-secreting cells. However, the role of SPL in insulin-secreting cells remains unknown.

Materials and methods: Insulin-secreting INS1E cells were transfected with the pcDNA3.1 SPL vector or with siRNA against SPL. Cells were treated with glucose (3, 10, 30 mM) and/or cytokines (IL-1 β 600 U/ml or with a cytokine mix: IL-1 β 60 U/ml, TNF α 185 U/ml and IFN γ 14 U/ml). After a 24 h incubation, gene expression (qRT-PCR), caspase-3 activation (Caspase-Glo 3/7 kit), ROS production (DCFDA oxidation assay), ATP (ATPlite luminescence assay) and glucose-stimulated insulin secretion (RIA) were determined.

Results: The measurement of SPL expression in several rat tissues and INS1E cells revealed a moderate SPL expression level in INS1E cells compared to different rat tissues. Cytokines induced caspase-3 activation in INS1E-control cells (IL-1 β 150% vs. cytokine mix 195%). This deleterious effect was significantly reduced by SPL-overexpression (IL-1 β 118% vs. cytokine mix 126%) and significantly enhanced by SPL-suppression (IL-1 β 302% vs. cytokine mix 292%). The protective effect of SPL-overexpression was associated with a reduced cytokine-induced ROS formation (INS1E-

control cells, IL-1 β 167%, cytokine mix 212%; INS1E-SPL cells, IL-1 β 132%, cytokine mix 144%). SPL-suppression increased cytokine-mediated ROS production (IL-1 β 252%, cytokine mix 290%). Interestingly, SPL-overexpression had a potentiating effect on glucose-stimulated insulin secretion (GSIS) (INS1E-control cells, 3 mM Glc 0.078, 10 mM Glc 0.206, 30 mM Glc 0.192; INS1E-SPL cells, 3 mM Glc 0.085, 10 mM Glc 0.450, 30 mM Glc 0.403 ng/ μ g DNA/h). The suppression of SPL weakened the stimulatory effect of glucose on insulin secretion (3 mM Glc 0.063, 10 mM Glc 0.071, 30 mM Glc 0.088 ng/ μ g DNA/h). Upon exposure to cytokines a significant 5-7 fold decrease in the amount of secreted insulin was observed in INS1E-control cells. The cytokine-mediated inhibition of glucose-induced insulin secretion was reduced by SPL-overexpression and potentiated by SPL-suppression. The effect of SPL on GSIS correlated with parallel changes in ATP content (INS1E-control cells, IL-1 β 85%, cytokine mix 63%; INS1E-SPL cells, IL-1 β 93%, cytokine mix 87%; INS1E-siRNA-SPL, IL-1 β 71%, cytokine mix 49%, vs. 100% of untreated cells).

Conclusion: This study demonstrates that SPL plays an important role in the regulation of beta cell function and the cytokine toxicity to insulin-secreting cells. It seems that the concentration of intracellular S1P, controlled by SPL, must be tightly regulated to maintain the proper beta cell function and to prevent cytokine-mediated toxicity.

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Unique effects of acute exposure to low concentrations of IL-1beta on primary beta cell function and gene expression

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Background and aims: The cytokine IL-1beta is thought to contribute to the low-grade systemic and islet inflammation leading to insulin secretion defects in type 2 diabetes. Indeed, longterm exposure (>24 h) of beta cells to very high concentrations (20 ng/mL) of IL-1beta results in modulation of gene expression by NF κ B, impaired function and ultimately cell death. Interestingly, IL-1beta can also exert beneficial effects on beta cells following exposure for >24 h to low concentrations of IL-1beta, with improved insulin secretion, increased proliferation and decreased apoptosis but the mechanisms are less well understood. We have observed previously that shorter exposure (2 h) to low (0.1 ng/mL) but not high (20 ng/mL) concentrations of IL-1beta enhances glucose-stimulated insulin secretion through focal adhesion and actin remodeling. We now study the mechanism of this beneficial action of IL-1beta on beta cell function.

Materials and methods: Rat primary beta cells or human islets established in monolayer on extracellular matrix were incubated for 1 h at 2.8 mmol/L followed by 1 h at 16.7 mmol/L glucose in the continued presence (or not, control) of 0.1 ng/mL or 20 ng/mL rat or human IL-1beta respectively. Insulin secretion was measured by radioimmunoassay. To inhibit transcription, rat beta cells were pre-treated 1 h with actinomycin D (0.1 μ g/mL). NF κ B (p65) was localized by immunofluorescence. RNA was extracted and sequenced by Illumina HiSeq to an average depth of 20M reads. Statistical analyses were performed in R and differential gene expression with DESeq2. Data are mean \pm SE \geq 3 independent observations.

Results: Glucose-stimulated insulin secretion (GSIS), but not basal secretion, of rat primary beta cells and human islets, was increased by 56.5 \pm 17.4% (p=0.032) and 74.9 \pm 20.5% (p=0.021) respectively by 0.1 ng/mL IL-1beta whereas 20 ng/mL was without effect. NF κ B was translocated rapidly to the nucleus during exposure of rat beta cells to 0.1 ng/mL IL-1beta and blockade of NF κ B activation by Bay 11-7082 (5 μ mol/L) prevented IL-1beta enhancement of GSIS. Furthermore, 0.1 ng/mL IL-1beta had no impact on GSIS in the presence of actinomycin D. Taken together these data suggested that IL-1beta enhanced GSIS through modulation of gene expression by NF κ B. Transcriptome analysis by RNA-seq revealed 928 genes differentially expressed by the low dose and 1738 by the high dose of IL-1beta with 55 up-regulated and 88 down-regulated uniquely by 0.1 ng/mL IL-1beta (p< 0.001). Of these genes modulated uniquely by low dose IL-1beta, 6.99% were classed as vesicle and secretory granule components, 13.3% are involved in the regulation of the transcription. The expression of one gene of particular interest for beta cell function, SIRT1, was increased from 8.52 \pm 0.35 to 10.28 \pm 0.54 RPKM (p=0.016) by 2 h exposure to 0.1 ng/mL IL-1beta with no significant change with 20 ng/mL.

Conclusion: Acute exposure of beta cells to 0.1 ng/ml IL-1beta can act rapidly and positively on insulin secretion through the regulation of gene transcription. Results obtained with RNA sequencing analysis revealed changes

in gene expression profiles elicited uniquely by acute exposure to low but not high concentrations of IL-1 β and that could drive improved beta cell function.

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The hepatokine fetuin-A triggers inflammation without inducing cell death but improves cAMP-dependent insulin secretion of pancreatic islets

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Background and aims: Nonalcoholic fatty liver displays an altered secretion of hepatokines. Previously, we found that increased plasma levels of the hepatokine fetuin-A strongly predict the incidence of type 2 diabetes in humans. As mechanisms of action, fetuin-A directly inhibits insulin receptor signaling and augments inflammation by interacting with fatty acids to activate TLR4. In addition, we found a negative relationship of fetuin-A levels with adjusted insulin secretion only in subjects with impaired glucose tolerance. The present study aims to examine the impact of fetuin-A on fatty acid-induced effects in pancreatic beta-cells.

Materials and methods: Human islets received from the European Centers of Islet Transplantation (ECIT) and mouse islets were cultured in medium supplemented with human fetuin-A or serum albumin as control (0.6 mg/ml each) for 48h. Due to the low concentration of albumin palmitic acid was used at a concentration of 60 μ mol/l, which does not induce metabolic stress. Changes in gene expression were analyzed by microarray and qRT-PCR. Apoptosis was estimated by TUNEL staining. Insulin secretion was evaluated after static incubation by radioimmunoassay.

Results: In agreement with previous observations in other cell types, fetuin-A increased 2-fold the mRNA levels of IL1 β , IL33, IL24 and IL17RB in human islets. The stimulation of cytokine production depended on the activation of TLR2/4, as fetuin-A elevated the mRNA amount of IL1 β and MCP-1 in WT, but not in TLR2/4 KO islets. On the contrary, palmitic acid neither stimulated nor augmented the fetuin-A-mediated cytokines production. Surprisingly, in spite of increasing the cytokine levels, fetuin-A failed to induce apoptosis and, moreover, significantly inhibited palmitic acid-induced cell death. In addition, chronic exposure to fetuin-A did not affect glucose and palmitic acid stimulated insulin secretion but it selectively and specifically improved forskolin and exendin-4 stimulation of secretion.

Conclusion: These results suggest that fetuin-A stimulates cytokine production in a TLR-dependent manner. However, it exerts rather beneficial effects on beta cell function, as it does not induce apoptosis and improves cAMP-amplified insulin secretion.

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PS 020 Clinical immunology of type 1 diabetes

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The promoters of genes may be the closest link between type 1 diabetes and other autoimmune diseases

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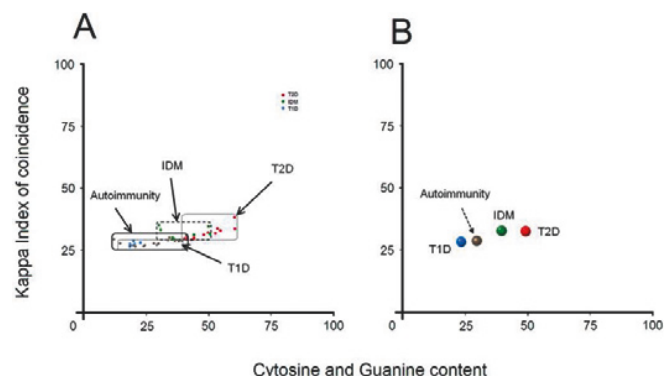
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Background and aims: Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease that selectively targets pancreatic beta cells. T1D is thought to arise from the progressive loss of beta cells occurring over months upto years. Despite extensive genetic studies in T1D, rarely analyzes were performed with respect to promoters of genes associated with autoimmunity and T1D.

Materials and methods: For our study we chose a set of representative genes for several diabetes phenotypes. We analyzed the promoters of genes associated with T1D, including HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DPB1, PTPN22, TLR7, CTLA4, GSDMB, STAT4, IL7R, C1QTNF6, CD55, CTSH, ERBB3 and INS and with T2D, including CAMK1D, DUSP9, HHEX, IRS1, MADD, NOTCH2, TP53INP1, VPS13C, WFS1, ZFAND6, HMG2, PPARG, CDKN2AIP, PROX1 and TCF7L2. In order to detect a possible relationship between T1D and other autoimmune diseases, we analyzed the promoters of genes associated with more than one autoimmune disease, including RGS1, IL10, AFF3, IL21, INS, KIF3A, GPR183, GSDMB, ORMDL3, PTPN2, FUT2, TLR7, PTPN22, CTLA4, CD55 and STAT4. The DNA pattern methodology that we have used, determines the coexpression relationships between genes. Thus, in the resulting distribution, the overlapping positions (or close positions) of promoters of these genes indicate that those promoters use transcription factors in common.

Results: Our distribution shows that there is a common denominator between promoters of genes involved in autoimmunity. Both the promoters of T1D genes and those associated with other autoimmune diseases occupy the same overlapping positions in our distribution. The figure (Figure A) below shows the general distribution of different phenotypes, namely T1D (in blue), T2D (in red), genes associated with both phenotypes (included in „Intermediary Diabetes Mellitus” - IDM phenotype) in green and promoters of genes associated with autoimmunity (in brown). In a general distribution of the average positions of gene promoters for each phenotype (Figure B), the close functional relationship between T1D and autoimmune diseases gene promoters is becoming increasingly clear. The clustering of these genes, very close to each other, suggests that transcription factors involved in their expression may be part of the triggering mechanism of anti beta cell autoimmunity. The close distribution of the promoters of genes associated with the analyzed phenotypes represents a functional correlation, in the sense that these genes are more likely to be co-expressed.

Conclusion: Promoters of genes associated with T1D and with other autoimmune diseases share one common factor in causing T1D, namely the transcription factors involved in long term gene expression. The wide distribution between promoters of genes associated with T1D and T2D strongly suggests that these two phenotypes are not using transcription factors in common.



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A novel autoantibody detected in patients with fulminant type 1 diabetes

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Background and aims: Fulminant type 1 diabetes (FT1D) is a subtype of type 1 diabetes characterized by complete insulin deficiency resulting from the destruction of pancreatic beta cells even at the disease onset. Massive cellular infiltration of T-cells and macrophages has been detected in islets and exocrine pancreas, suggesting that immune disorder might contribute to the development of FT1D. However, islet-related autoantibodies, such as GAD Ab or IA-2 Ab, were usually negative, indicating that we have no useful biomarker to diagnose FT1D. Therefore, we performed serological antibody analysis cyclopedically to discover a novel diagnostic marker of FT1D.

Materials and methods: First, we analyzed a total of 6 serum samples from 3 patients with FT1D (1 sample in acute and 1 in chronic phases from each patient) on over 9000 human protein arrays (Invitrogen ProtoArray[®] Human Protein Microarray v5.0) by fluorescence. Second, titres of the antibody were measured in sera from 20 patients with FT1D (both in acute and chronic phases), 32 patients with type 1A diabetes (T1AD), 30 patients with type 2 diabetes (T2D), 22 patients with autoimmune thyroid disease (AITD) and 30 healthy control subjects (HC) using ELISA assay. Duration of FT1D in acute and chronic phase was 8.1 ± 6.3 (mean \pm SD, day) and 24.2 ± 9.1 , respectively.

Results: By serological antibody analysis of over 9000 antibodies, we detected 8 antibodies which showed high signals from all 3 patients with FT1D in acute phase (acute/chronic phase ratio >1.4). We focused on one novel antibody that has not yet been reported in any conditions and measured its titre by ELISA assay. Titres of the antibody were 0.1071 ± 0.0374 (mean \pm SD, arbitrary unit) in FT1D patients (acute phase), 0.0823 ± 0.0182 in FT1D patients (chronic phase), 0.0771 ± 0.0171 in T1AD patients, 0.0710 ± 0.0171 in T2D patients, 0.0701 ± 0.0116 in AITD patients and 0.0641 ± 0.0078 in HC. Significantly high titre of the antibody was detected in sera from FT1D patients in acute phase (versus T1AD patients; $P=0.0002$, versus T2D patients; $P<0.0001$, versus AITD patients; $P<0.0001$, versus HC; $P<0.0001$), and also in sera from FT1D patients in chronic phase (versus T1AD patients; $P=0.0188$, versus T2D patients; $P=0.0093$, versus AITD patients; $P=0.0229$, versus HC; $P=0.0027$). Titres of the antibody were higher from FT1D patients in acute phase than those in chronic phase ($P=0.0023$, paired t-test). There was no significant correlation between the titres of the antibody and age, gender, HbA1c, plasma glucose and GAD or IA-2 antibody level in all diabetic patients.

Conclusion: We detected one novel antibody whose titre was high in sera from patients with FT1D both in acute and chronic phases. This antibody might become a diagnostic marker of FT1D.

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Inflammatory profile in patients with type 1 diabetes: exploring a hidden side of type 1 diabetes

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Background and aims: The aims of this study are to compare inflammatory markers (IL-6, TNF α , high sensitivity C reactive protein -hsCRP-, TNF-like weak inducer of apoptosis -sTWEAK-, and scavenger receptor CD163 -sCD163-) between 158 T1DM patients and 638 healthy subjects and to evaluate if there are differences in these inflammatory markers in subjects with T1DM according to glycaemic control measured by HbA1c levels, to the type of insulin regimen (multiple daily injections [MDI] vs. continuous subcutaneous insulin infusion [CSII]), the presence of microvascular complications, the quality of life, the distress, and the symptoms of depression.

Materials and methods: Cross-sectional study which involved 158 patients with T1DM attending our diabetes unit. Additionally, 638 healthy subjects from the di@bet.es study (a cross sectional study comprising a representative sample of the Spanish adult population) and the Pizarra study (a population based cohort study undertaken in Pizarra, Spain) with similar age, sex distribution and BMI than the study patients were selected. Sociodemographic, clinical and biochemical data (HbA1c and lipid profile) were recorded. Quality of life was evaluated by the Diabetes Quality of Life questionnaire (DQoL), distress was assessed by the Diabetes Distress Scale (DDS), and symptoms of depression was evaluated by the Beck Depression Inventory (BDI). Inflammatory markers including IL-6, hs-CRP, TNF α , sTWEAK and sCD163 were measured.

Results: a) T1DM patients vs. healthy subjects. Patients with T1DM had lower sTWEAK levels (806 ± 1312 vs. 1040 ± 1212 pg/mL, $p=0.0001$) and higher IL-6 levels (2.6 ± 3.0 vs. 1.7 ± 2.6 pg/mL, $p=0.001$) and hsCRP levels (3.4 ± 6.3 vs. 3.0 ± 5.2 mg/L, $p=0.001$) than healthy subjects with similar age, sex and BMI. No significant differences were found in TNF α and sCD163 levels. b) T1DM patients. Patients with T1DM had significantly higher IL-6 levels if they were treated with MDI vs. CSII (2.9 ± 3.3 vs. 1.5 ± 1.4 pg/mL, $p=0.017$), had microvascular complications (3.8 ± 3.5 vs. 2.2 ± 2.8 pg/mL, $p=0.006$) and had symptoms of depression (3.5 ± 3.7 vs. 2.2 ± 2.6 pg/mL, $p=0.023$). If we compare patients treated with CSII with those receiving MDI, the former ones had significantly lower HbA1c levels (7.5 ± 0.9 vs. $7.9 \pm 1.3\%$, $p=0.04$), lower score in BDI (7.5 ± 8.8 vs. 12.2 ± 12.2 , $p=0.008$), DDS (1.5 ± 0.8 vs. 1.9 ± 1.0 , $p=0.05$) and DQoL (83.4 ± 19.0 vs. 90.8 ± 22.0 , $p=0.03$), lower IL-6 levels and higher TNF α levels (4.6 ± 8.5 vs. 1.5 ± 0.82 pg/mL, $p=0.001$), adjusted for age, sex and BMI.

Conclusion: Patients with T1DM had a different inflammatory pattern than their healthy counterparts. IL-6 levels were increased in patients with T1DM receiving MDI insulin regimen, with microvascular complications, and with symptoms of depression.

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Circulating immune mediators are closely linked in the early course of adult onset type 1 diabetes

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Background and aims: Relationships between circulating immune mediators (cytokines, chemokines and growth factors) and a beta cell destructive autoimmune process are poorly elucidated in adult-onset type 1 diabetes. We measured serum levels of immune mediators in such patients with newly diagnosed disease during the course of ongoing deterioration of insulin secretion.

Materials and methods: Levels of 27 immune mediators were measured in 34 GADA (glutamic acid decarboxylase antibodies) positive type 1 diabetic patients, aged 27.4 ± 1.2 years at a mean of 7 weeks after diagnosis (designated 0 month) and 6 months later. Endogenous insulin secretion was assessed by C-peptide glucagon stimulation tests at 0, 6 and 12 months. Supplementary data were obtained in 9 GADA positive type 1 diabetic subjects and in 43 non-diabetic age and sex matched subjects. Results were analyzed with and without adjustment for body weight (BMI).

Results: Mean levels of stimulated C-peptide had declined by 10% after 6 months and by 28% after 12 months. This decline was not reflected by levels of immune mediators which displayed large inter- but small intra-individual differences with only minor changes observed between measurements at 0 and at 6 months (p -values for correlations ranging from 0.000 to 0.005). Levels of the majority of immune mediators were strongly and positively correlated to each other in the diabetic subjects. However, such correlations were also found in the non-diabetic subjects and data did not suggest any network of immune mediators specific for the diabetic subjects. Body weight (BMI) was positively associated with levels of IL-1 α , IL-2, IL-4, IL-6, IL-17, Basic FGF, GCSF, IFN gamma and MIP-1 α . When adjusted for BMI, levels at 0 month for Basic FGF and MIP-1 α were inversely associated with the percentage decline in stimulated C-peptide from 0 to 12 months (nominally $p<0.05$).

Conclusion: 1) There are close associations between different immune mediators 2) These associations are not specific for autoimmune diabetes 3) BMI is a major confounder 4) The associations of beta cell decline to individual immune mediators need confirmation in further studies.

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Relationship between autoantibodies combination, metabolic syndrome and its components in autoimmune diabetes in adults

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Background and aims: Although clinical presentation of latent autoimmune diabetes in adults (LADA) at diagnosis frequently features clinical traits of type 2 diabetes mellitus (T2DM), it is the autoimmune process that drives LADA patients through slower beta-cells destruction and insulin dependency within few years. Majority of LADA patients has pronounced positivity for glutamic acid decarboxylase enzyme antibodies (GAD-Ab) frequently accompanied by moderate levels of circulating autoantibodies to islet cell cytoplasmic antigens (ICA-Ab). Positivity for tyrosine phosphatase-like transmembrane glycoprotein (IA2-Ab) has also been reported but to a lesser extent. While simultaneous positivity for ICA-Ab, and GAD-Ab with eventual presence and of IA2-Ab indicate patients with insulin deficiency and typical type 1 LADA phenotype, GAD antibody positivity, even in higher titers, represent a marker for slower progressing LADA. The role of IA2Ab positivity in LADA phenotypisation is poorly understood and controversial. The aim of our study was to establish a possible association between autoimmune profile of patients regarding double or triple antibody positivity and LADA phenotype in the context of metabolic syndrome (MS) prevalence, individual components of metabolic syndrome and chronic diabetic complications.

Materials and methods: This cross-sectional study population comprised 69 islet cell antibody positive patients coming for their comprehensive annual review. Rate of positivity was calculated by determining end-point titres of samples that were converted to the units of Juvenile Diabetes Foundation (JDF-U) by comparison with a standard curve of end-point titer of standard sera. The threshold of detection was >5 JDF units. GAD and IA2-Abs were detected with an enzyme-linked immunosorbent assay (Euroimmun AG, Luebeck, Germany) and the results were expressed in arbitrary units. The cut-off limit was 10 U/ml for GAD Abs and 15 U/ml for IA2-Abs. MS was defined according to the International Diabetes Federation definition, arterial hypertension (AH) was considered as blood pressure > 130/85 mmHg or the use of antihypertensive drugs. Patients were divided into three groups: Gad Ab only positive (n=28), GAD Ab+ICA Ab positive (n=26) and GAD Ab+ICA Ab+IA2 Ab positive (n=15).

Results: Twenty five (36.2%) patients were male, mean age approximately 51 years with disease duration 8 years. The lowest value of waist circumference (80 vs 82 vs 89.5 cm), MS (1 vs 11 vs 15) and AH (1 vs 11 vs 16) prevalence was found in the group positive for all three antibodies compared the others two groups. In the multinomial multivariate logistic regression model higher waist circumference (OR 0.931 (0.869-0.988)), MS prevalence (OR 0.062 (0.007-0.537)) and AH prevalence (OR 0.065 (0.007-0.577)) were negatively associated with triple Ab positivity compared to single Ab positivity, and compared to double Ab positive group with ORs as follows: 0.940 (0.877-0.990), 0.097 (0.011-0.855), and 0.099 (0.011-0.879).

Conclusion: Our results highlight the importance of the inverse association of simultaneous Abs positivity for ICA, GAD and IA2 with the presence of MS and its components in LADA patients. This inverse relationship might implicate the presence of LADA patients' phenotype closer to type 1 diabetes.

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Autoimmune gastrointestinal markers in patients with type 1 diabetes

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Background and aims: In patients with type 1 diabetes the prevalence of other autoimmune diseases is 3 to 5 fold higher than in non-diabetic patients. Gastrointestinal autoimmune diseases are generally asymptomatic up to an advanced stage when they become major causes of mortality if not di-

agnosed and treated on time. The presence of immunological markers may be indicative of gastrointestinal autoimmune diseases even in a clinically asymptomatic case. The aim of our study was to investigate the frequency of specific autoantibodies occurrence in patients with T1 DM, to clarify the role of polyautoimmunity in the appearance of autoantibodies and the association of autoantibody titer with clinical features of accompanying autoimmune diseases.

Materials and methods: 84 patients with T1DM and 21 healthy subjects (control group) were included in the study. T1DM patients were divided into two groups. The first group included 58 patients with T1DM only. The second group consisted of 26 patients with T1DM and another autoimmune disease (thyroid, celiac and Addison's disease, rheumatoid arthritis, vitiligo, myasthenia gravis). Anti-gastric parietal cells antibodies (AGPA) - markers of autoimmune gastritis, antinuclear antibodies (ANA), and anti-smooth muscle antibodies (ASMA) - markers of autoimmune hepatitis, antimitochondrial antibodies (AMA) - primary biliary cirrhosis marker were detected by using indirect immunofluorescence antibody test. All subjects were tested for ICA, GADA, IA-2A, C-peptide, HbA1c. Gastroscopy and ultrasound liver examination were performed to every patient.

Results: Significant difference was observed in frequency of ANA, AGPA, ASMA occurrence between the study groups and control group, $p < 0.05$ (table 1). Significantly higher appearance of ANA was observed in patients of the 2nd group compared to the 1st group ($p = 0.007$). 50% of patients with gastritis confirmed by gastroscopy had AGPA. 13% of patients with ultrasound signs of hepatic abnormalities were positive to ANA, 14% - to ASMA, 21% - to AMA compared to 28% ($p = 0.39$), 38% ($p = 0.15$) and 19% ($p = 0.86$) of patients with normal hepatic features respectively.

Conclusion: Our study showed a high frequency AGPA, ANA, ASMA, AMA occurrence in patients with T1 DM. This data indicates the increased risk of gastric and hepatic autoimmune diseases in T1DM patients. Only ANA had significantly higher appearance in patients with several autoimmune diseases. This might be due to the fact that ANA is a less specific autoantibody and can cause less specific autoimmunity against several organs. Other antibodies were observed with the same frequency in both study groups ($p < 0.05$) but ANA, AGPA, ASMA had significantly higher appearance in patients with diabetes compared to the control group. These findings provide a rationale for screening, early diagnosis and treatment of additional autoimmune diseases in T1DM patients.

Table 1. Autoantibody occurrence in study groups and control group

Autoantibody	1st group	2nd group	Control group	p 1-2	p 1-3	p 2-3
ANA	12/26=46	9/58=16	1/21=5	0,007	0,005	0,36
AGPA	11/26=42	23/58=41	0/21=0	0,89	0,002	0,001
ASMA	10/26=38	18/58=32	1/21=5	0,75	0,018	0,029
AMA	5/26=19	11/58=20	1/21=5	0,79	0,29	0,21

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Leukocyte profiles differ between type 1 and type 2 diabetes and are associated with metabolic phenotypes. Results from the German diabetes study (GDS)

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Background and aims: Altered immune reactivity precedes and accompanies type 1 and type 2 diabetes. We hypothesized that the metabolic phenotype relates to the systemic cellular immune status.

Materials and methods: A total of 194 metabolically well controlled patients with type 1 diabetes (n=62, mean diabetes duration: 1.29 years) or type 2 diabetes (n=132, 1.98 years) and 60 normoglycemic persons underwent blood sampling for automated white blood cell counting (WBC) and flow cytometry. Whole body insulin sensitivity was measured with hyperinsulinemic-euglycemic clamp tests. Analysis of covariance was performed to compare groups and Spearman's non-parametric correlations and partial correlations adjusted for age, sex, and BMI were estimated.

Results: Patients with type 2 diabetes had higher WBC counts than controls ($P < 0.001$) along with higher percentage of T-cells ($P = 0.038$), activated T helper (Th)- ($P < 0.001$) and cytotoxic T (Tc)-cells ($P < 0.001$), but lower pro-

portions of natural killer (NK) cells ($P<0.05$). In type 1 diabetes, percentage of activated Th- ($P<0.001$) and Tc-cells ($P=0.006$) were also higher compared to controls, whereas the ratio of regulatory T (Treg)-cells to activated Th-cells was lower suggesting diminished regulatory capacity. Parameters of glycaemic control (HbA1c, blood glucose) related positively to Treg-cells only in type 2 diabetes ($P<0.05$). Upon age-, sex-, and body mass-adjustments, insulin sensitivity correlated positively with monocytes ($r=0.469$, $P<0.01$), while circulating lipids correlated positively with T-cell subsets in type 1 diabetes ($r=0.281$, $P<0.05$).

Conclusion: Immune cell phenotypes showed distinct frequencies of occurrence in both diabetes types, and associate with insulin sensitivity, glycemia and lipidemia.

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Insulin-induced level of TNF- α : the early marker of type 1 diabetes mellitus

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Background and aims: To investigate an influence of monoclonal activator - insulin upon cytokine-producing function of peripheral blood cells in the patients with type 1 and 2 diabetes mellitus.

Materials and methods: 25 type 1 diabetes mellitus patients and 9 patients with type 2 diabetes mellitus were examined. As a control we examined 10 healthy persons. Mononuclear leukocytes were isolated by centrifugation in the ficoll-verographin density gradient. The cells thus obtained were resuspended in the complete nutritient medium reducing their concentration to 2.0×10^6 /ml. Phytohemagglutinin (PHA-P, Sigma) (10 mcg/ml) and insulin (insulin human, Sigma) (10 mcg/ml) were added to the samples to stimulate mononuclear leukocytes; cell suspensions were further incubated for 36 hours. Initial, PHA-induced and insulin-induced levels of interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, gamma interferon (IFN- γ) and tumor necrosis factor alpha (TNF- α) in supernatants of cell cultures were measured by solid phase immunoassay at 450 nm. Results were compared with clinical characteristics of the patients. There were 8 males and 17 females in the group 1 and 2 males and 7 females in the group 2. Median age, diabetes mellitus duration, HbA1c in the group 1 and 2 were respectively 32 years, 17 years, 8,3 % and 63 years, 12 years, 9,1 %. Intensities of microvascular complication (diabetic retinopathy and diabetic nephropathy) were higher in the group 1. 8 patients (32%) from group 1 had diabetic retinopathy on the last stage and only 1 patient (11%) from the group 2 had diabetic retinopathy on the same stage. Only 1 patient (11%) from the group 2 had diabetic nephropathy on microalbuminuria stage and 7 patients from the group 1 (28%) had diabetic nephropathy on proteinuria stage and on the stage of renal failor.

Results: For estimation of immune response intensity we used for the first time the index of autoimmune inflammation (IAI) - correlation of insulin-induced and basal production of TNF- α . The T-cell response on insulin was absent in control group and in patients with type 2 diabetes mellitus. Only one patient with type 2 diabetes mellitus had IAI more than 1. It was the basis for a change the diagnosis from type 2 diabetes to type 1 diabetes mellitus. Our patients with type 1 diabetes mellitus had IAI from 3,09 till 19,23. It correlated with the stage and intensities of microvascular complications (diabetic retinopathy and diabetic nephropathy) of these patients.

Conclusion: The TNF- α production ratio of insulin-stimulated versus resting cells may be used as a reliable early marker of autoimmune versions of diabetes mellitus, marker of autoimmune process activity in pancreatic tissue and disease severity in type 1 diabetic patients.

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Regulatory T cell dysfunction in type 1 diabetes

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Background and aims: Type 1 diabetes mellitus (T1DM) develops as a result of the autoimmune damage of pancreatic β -cells. Regulatory T-cells (Treg) have a crucial role in limiting the naturally occurring autoreactivity. Foxp3 is a master regulator transcription factor of Treg differentiation. Active Treg cells express high levels of IL-2 receptor α -chain (CD25) and CTLA4 (Cytotoxic T-Lymphocyte Antigen 4). The aim of our study was to clarify certain details of the immunological dysregulation and Treg dysfunction.

Materials and methods: Forty patients with T1DM (M/F= 19/21; 33.7 ± 10.04 years) and forty age-matched non-diabetic control subjects (M/F=16/24; 32.97 ± 11.63 years;) were involved. The mean disease duration was $15.76 (\pm 7.23)$ years. Fasting, EDTA-anticoagulated blood samples were collected. CD3, CD4, CD8, and CD25 surface proteins, intracellular CTLA4 and intranuclear Foxp3 were stained with specific fluorescent antibodies. A Beckman Coulter Navios flow cytometer and Kaluza software were used for quantitative analysis. Fluorescence intensity was described as median fluorescence intensity (MFI). Shapiro-Wilks normality test, Mann-Whitney-U-test, T-test and the Statsoft Statistica software were used.

Results: The proportion of Foxp3+ cells among CD4+ lymphocytes was not different in T1DM patients and control subjects. The proportion of CD25+ cells among CD4+Foxp3+ lymphocytes was lower in T1DM patients ($72.89 \pm 7.74\%$ vs. $63.23 \pm 12\%$, $p<0.0001$). CD25 and CTLA4 expression (MFI) of T-lymphocytes was significantly lower in T1DM patients (CD25: 0.2 ± 0.21 vs. 0.38 ± 0.33 , $p<0.01$; CTLA4: 1.02 ± 0.45 vs. 1.3 ± 0.48 , $p<0.05$). Positive correlation was observed between the CD25 and the CTLA4 expression of T-lymphocytes ($p<0.0001$; $r=0.57$).

Conclusion: Although the CD4+Foxp3+ Treg cell population was not quantitatively different, the higher proportion of CD4+Foxp3+CD25- cells and lower CTLA4 expression in T-lymphocytes may reflect an increased proportion of the inactive Treg cell subpopulation. This might contribute to the impaired immune regulation. To overcome the impaired Treg cell activation in T1DM might be an attractive therapeutic approach in the future.

PS 021 Measuring and preserving insulin secretion in type 1 diabetes

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¹¹¹In-exendin uptake in the pancreas correlates with the beta cell mass but not with the alpha cell mass

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Background and aims: A method to non-invasively determine the beta cell mass in vivo would enable us to study the pathophysiology of diabetes and is still a major unmet need. Targeting of the GLP-1R with ¹¹¹In-labeled exendin is an attractive approach for determination of the beta cell mass and preclinical studies as well as a proof-of-concept study in type 1 diabetic patients and healthy subjects showed a direct correlation of beta cell mass and radiotracer uptake. Despite these highly promising initial results, the influence of alpha cells on the uptake of the radiotracer remains a matter of debate. In this study we examined the specificity of ¹¹¹In-exendin in a rat model for beta cell loss by comparing the uptake of the tracer with the beta and alpha cell mass.

Materials and methods: Brown Norway rats were treated with 45 or 60 mg/kg alloxan (n=4 per group) in order to destroy the beta cells and 4 rats were injected with vehicle as a control. One week after injection of alloxan, 15 MBq ¹¹¹In-exendin (corresponding to 0.1 µg exendin) was injected and the pancreas was dissected 1 h after injection of ¹¹¹In-exendin. The radioactivity concentration was measured in a gamma counter and the beta cell and alpha cell mass were determined by morphometric analysis after immunohistochemical staining for insulin and glucagon, respectively.

Results: The uptake of ¹¹¹In-exendin (percentage of the injected dose) showed a strong positive linear correlation with the beta cell mass (Pearson $r = 0.90$). The absolute alpha cell mass was similar in healthy rats (2.2 ± 0.4 mg) and rats treated with 45 or 60 mg/kg alloxan (2.3 ± 0.3 mg and 1.8 ± 0.3 mg, respectively) and there was no significant correlation between the alpha cell mass and the uptake of ¹¹¹In-exendin (Pearson $r = 0.31$). The total mass of the endocrine pancreas was reduced in the alloxan treated rats (8.5 ± 1.9 mg, 5.5 ± 1.7 mg, and 2.8 ± 0.8 mg for healthy, 45 and 60 mg/kg alloxan, respectively) and percentage of glucagon positive cells of the total endocrine mass was increased after alloxan treatment ($26\% \pm 4\%$, $43\% \pm 8\%$, and $69\% \pm 21\%$ alpha cells of the total endocrine mass for healthy, 45 and 60 mg/kg alloxan, respectively). The uptake of ¹¹¹In-exendin showed a negative linear correlation with the alpha cell fraction (calculated by dividing the alpha cell mass by the total endocrine mass, Pearson $r = -0.81$).

Conclusion: The uptake of ¹¹¹In-exendin correlated with the beta cell mass, but not with the alpha cell mass. Together with the increased relative alpha cell mass and the negative linear correlation between the ¹¹¹In-exendin uptake and alpha cell fraction, these data clearly indicate towards specificity of ¹¹¹In-exendin to beta cells and that the influence of the alpha cells on ¹¹¹In-exendin uptake is negligible.

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Towards clinical PET imaging of pancreatic beta cells with [¹⁸F]exendin-4

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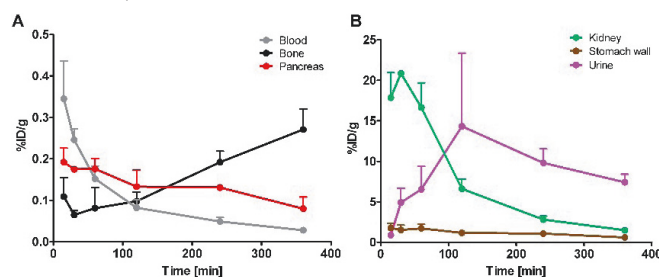
Background and aims: [¹⁸F]exendin-4 is a radioligand specific for the glucagon like peptide-1 receptor (GLP-1R) abundantly expressed in beta cells. Quantitative non-invasive PET (positron emission tomography) imaging of pancreatic beta cells would serve as a valuable diagnostic tool and provide means to monitor therapeutic interventions. Several exendin based tracers have been intensively explored for beta cell imaging. The obstacle with these has been high uptake in the kidneys. The aim of this study is to develop a novel [¹⁸F]exendin-4 tracer for clinical imaging of beta cells with PET.

Materials and methods: Biodistribution and kinetics of [¹⁸F]exendin-4 was evaluated in Sprague-Dawley rats (N=3–9 per time point) weighing 250–300 g. After intravenous injection of [¹⁸F]exendin-4 (19 ± 3 MBq/kg, mass 0.3 ± 0.2 nmol/kg) rats were sacrificed at 15 min, 30 min, 1 h, 2 h, 4 h or 6 h. The

organ-specific radioactivity was reported as a percentage of the injected dose per gram of tissue (%ID/g). The GLP-1R specificity was assessed using cold exendin-3 (N=1). Intrapancratic distribution of radioactivity was assessed using autoradiography. Islet labelling was verified by immunohistochemistry and islet-to-exocrine tissue ratios were analysed. Radioactive metabolites were determined by HPLC. For PET scans, rats were imaged up to six hours (dynamic 0–1 h, static 3.5–4 h and static 5.30–6 h) using Inveon Multimodality PET/CT.

Results: Radioactivity was nearly constant in the pancreas over the course of the study (0.18 ± 0.03 %ID/g at 1 h and 0.08 ± 0.03 %ID/g at 6 h p.i., Fig. 1A). Blocking studies indicated GLP-1R specific uptake in the islets. Autoradiography analysis of pancreatic sections showed that the islet-to-exocrine tissue ratio was 78 ± 29 at 1 h p.i. The amount of unchanged tracer in plasma was 28.3 ± 3.5 % at 1 h p.i. In line with other exendin based tracers, [¹⁸F]exendin-4 uptake by kidneys was high at 1 hour time point (16.7 ± 3.0 %ID/g), but thereafter its clearance was fast and retention decreased (1.5 ± 0.4 %ID/g, at 6 h p.i., Fig. 1B). After 6 h PET imaging, the highest tracer uptake was found in kidney, lung and stomach wall. Radioactivity in bone was low, indicating low defluorination of the tracer.

Conclusion: In conclusion, we found a specific and sustained uptake of [¹⁸F]exendin-4 in the pancreatic islets and high renal clearance of the tracer. These indications are promising for the development of novel [¹⁸F]exendin-4 towards clinical imaging of beta cells. Currently we are investigating [¹⁸F]exendin-4 biodistribution and pancreatic uptake in larger animals. Figure 1. Biodistribution of [¹⁸F]exendin-4 radioactivity in rat at various time points after tracer injection.



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¹¹¹In-exendin imaging in patients with type 1 diabetes and healthy controls

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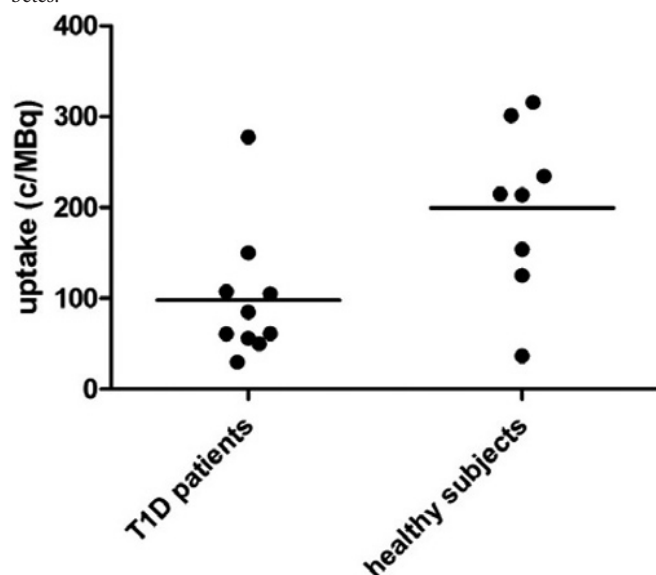
Background and aims: Currently the beta cell mass (BCM) can, in contrast to beta cell function, not be measured non-invasively. A non-invasive test would help to increase our knowledge about changes in beta cell mass during the development and treatment of diabetes. Preclinical studies showed that the pancreatic uptake of ¹¹¹In-labeled exendin, which targets to the glucagon-like peptide-1 (GLP-1) receptor, directly correlates with BCM. As a first step into clinical use of ¹¹¹In-exendin imaging for beta cell quantification, we acquired and analyzed single photon emission computed tomography (SPECT) images after ¹¹¹In-exendin administration in T1D patients and healthy subjects.

Materials and methods: Patients with long standing T1D (BMI below 27, age 21–60 yr, minimal 5 year duration of T1D) and healthy controls (normal glucose tolerance measured by OGTT, matched for age, gender and BMI) were eligible for the study. Four, 24 and 48 hours after i.v. injection of 150 MBq ¹¹¹In-Exendin-4, SPECT images were acquired for quantitative assessment of the uptake into the pancreatic beta cells. The pancreas volume was measured by CT. Uptake calculation was based on the counts within two spherical volumes of interest (VOIs) placed in the head and corpus region, multiplied by the pancreas volume. Counts were corrected for administered activity and time after injection, leading to the pancreatic uptake in counts per MBq (c/MBq).

Results: To date, 10 T1D patients and 8 healthy volunteers were included. The T1D patients showed an average uptake of 451 c/MBq in the whole pancreas

(SD 426 c/MBq). The average uptake of the healthy controls was more than two and a half times higher; 1258 c/MBq per pancreas (SD 645 c/MBq). Both groups showed large interindividual differences: the uptake in T1D patients ranges from 81 to 1445 c/MBq per pancreas and in the healthy subjects from 264 to 1998 c/MBq per pancreas. (See figure 1) Also the concentration of ^{111}In -Exendin in the pancreas (not corrected for pancreas size) is more than two times lower in the group with T1D. The images and subsequent quantitative analysis show that the ^{111}In retention in the pancreas remains stable for at least 4 to 48 hours after injection in the pancreas.

Conclusion: This clinical study indicates that ^{111}In -Exendin imaging with SPECT could indeed be the first technology to enable non-invasive beta cell quantification. In line with literature, it visualizes considerable interindividual differences in both groups and shows a, in general, much lower uptake of ^{111}In -Exendin in T1D patients than in healthy volunteers. Therefore it could be a valuable tool for further elucidating the complex pathophysiology in diabetes.



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Glucagon and mixed-meal tests to estimate beta cell and incretin functions in normals: relevance for studies in type 1 diabetes

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Background and aims: Measurement of C-peptide after the administration of different stimuli is the only instrument able to directly measure in vivo beta cell function and it is used as primary endpoint in several immunointervention trials. The two tests currently used are Glucagon Stimulation Test (GST) and Mixed Meal Tolerance Test (MMTT).

Materials and methods: We carried out a study in 10 healthy subjects (25-40 yrs) to evaluate beta cell function, Gastric Inhibitory Peptide (GIP) and Glucagon Like Peptide 1 (GLP1) which may affect C-peptide response to the two tests. GST and MMTT were carried out one week apart according to standard tests.

Results: At the end of GST (20 min) stimulated C-peptide showed a mean increase from baseline of 147.1% while at the end of MMTT (120 min), the mean increase of C-peptide was equal to 99.8% (Δ increase= 47.2%) while Max C-peptide reached during MMTT was greater than that gained during GST (C-pept maxMMTT=2.3nmol/L vs C-pep maxGST=1.9nmol/L). A positive and linear correlation was found between the incremental AUC (iAUC) C-peptide in GST and iAUC C-peptide in MMTT ($r=0.61$, $p=0.05$). These data show that the two tests can be considered equivalent in assessing beta cell function although there are differences: in MMTT, but not in GST, the incretin response affects C-peptide levels. A positive and linear correlation between GIP and C-peptide levels was found during MMTT ($r=0.92$,

$p=0.008$) and a positive and exponential correlation was found between GIP and insulin during MMTT ($R^2=0.82$). This was not the case with GST. No correlation was found between GLP-1 and C-peptide levels during MMTT and between GIP or GLP1 levels and C-peptide during GST.

Conclusion: Although the two stimulation tests may produce a similar response in C-peptide, they diverge in that the beta cell response to GST test is independent of the incretin axis whereas this is not the case with MMTT. This result indicates the two tests differ in their mechanisms of action of stimulating C-peptide secretion.

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Functioning beta cells in type 1 diabetes may not be as low as it is presumed

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Background and aims: Enhancing endogenous insulin production in type 1 diabetic patients (T1DP) can improve glycemic control and decrease complications and rates of mortality. However, it can be successful even if sufficient β -cell function is present. We aimed to evaluate the extent of β -cell function by determining fasting levels of C-peptide and those after meal stimulus.

Materials and methods: Sixtythree T1DP were enrolled. Ethics committee of our hospital approved the study protocol, which was in accordance with the Helsinki Declaration. Fasting C-peptide levels of all participants and stimulated (at 90 th minute post mixed meal) C-peptide levels of 54 were measured by using an electrochemiluminescence assay. Two categorizations were done using fasting (the first categorization) and at 90th minute post mixed meal test (the second categorization) of C-peptide levels. For the first categorization; the groups were classified as follows: patients with undetectable ≤ 0.1 ng/mL (group 1); with minimal 0.1-0.8 ng/mL (group 2); and with sustained ≥ 0.8 ng/mL (group 3) C-peptide levels. For the second categorization, groups were as follows: patients with undetectable ≤ 0.1 ng/mL (group 1); with minimal 0.1-0.8 ng/mL (group 2); and with sustained ≥ 0.8 ng/mL (group 3) C-peptide levels which increased at the 90th minute after the meal $\geq 150\%$ of fasting C-peptide level.

Results: For the first category; 25.4%, 49.2%, 25.4% of T1DP were in group 1, group 2 and group 3, respectively. For the second category; 22.2% of T1DP were in group 1, 24.1% of them in group 2 and 53.7 % of them in group 3. For the first categorization 74% and for the second categorization 77.8% of T1DP had detectable C-peptide levels. More than 50% of T1DP had a response to meal stimulus with C-peptide levels ≥ 0.8 ng/mL which increased up to $\geq 150\%$ of fasting C-peptide levels like non-diabetics.

Conclusion: The finding that presence of insulin secretion which increases after meals, suggests the possibility of undergoing β -cell regeneration within many T1DP.

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Residual beta cell function in long-standing childhood onset type 1 diabetes

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Background and aims: Some people with type 1 diabetes have functioning beta cells years after diagnosis, but the reason is unclear. We aimed to determine the frequency of residual C-peptide secretion in people with long-standing type 1 diabetes recruited to the Bart's-Oxford study at diagnosis and relate this to current and baseline islet autoantibodies.

Materials and methods: We measured two-hour post-meal urine C-peptide:creatinine ratio (UCPCR) and autoantibodies to glutamate decarboxylase (GADA) and islet antigen-2 (IA2A) in samples collected from 140 patients (median age at diagnosis 11.9 years, 50% male) a median of 22 years

(range 12.2–28 years) after diagnosis. Baseline status for GADA, IA2A and zinc transporter 8 autoantibodies was determined in samples collected within 24 months of diagnosis (median 33 days). UCPCR thresholds equivalent to mixed meal-stimulated serum C-peptide ≥ 0.2 nmol/L and ≥ 0.03 nmol/L were used to define 'preserved' and 'minimal' endogenous insulin secretion. HLA class II genotype was established by PCR-SSP. Associations were examined by chi-squared and non-parametric testing.

Results: Of the 140 patients, 23 (16.4%) still had detectable endogenous insulin secretion; seven (5.0%) 'preserved' and 16 (11.4%) 'minimal'. 62% of participants had at least one antibody in current samples; 29% were GADA+ and 50% IA2A+. Persistent C-peptide secretion was inversely related to age at diagnosis ($p=0.0009$), and no one diagnosed before age 10 had 'preserved' secretion. UCPCR was independent of diabetes duration and baseline or current autoantibody status. Of 7 individuals with 'preserved' endogenous insulin secretion, 6 had GADA at diagnosis with at least one diabetes risk-associated HLA class II haplotype.

Conclusion: A subset of patients with proven autoimmune-mediated type 1 diabetes has endogenous insulin secretion many years after diagnosis, but this is rarely found in individuals diagnosed in early childhood. Ongoing islet autoimmunity is also common. We hypothesise that, while aggressive early onset autoimmunity results in complete beta cell destruction, the less aggressive autoimmune process associated with later onset type 1 diabetes allows residual beta cells to provide a focus for persistent function as a result of regulated autoimmunity and/or beta cell renewal.

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High GADA titer significantly increases the risk of insulin requirement in LADA: a 7-year follow-up (NIRAD Study 7)

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Background and aims: The aim of the study was to determine whether glutamic acid decarboxylase antibody (GADA) titer and other clinical parameters could define the risk of progression to insulin therapy in LADA patients during a 7-years follow-up.

Materials and methods: N= 222 LADA and n=430 type 2 diabetes subjects were followed for 7-years from the time of GADA screening to evaluate their progression towards insulin therapy. Kaplan-Meier curves and multivariate logistic regression analysis were performed to identify markers able to influence this progression.

Results: During the follow-up, drop out was 4% in both groups. N=119 (56.1%) out of n=212 LADA and n=86 (20.9%) out of n=412 type 2 diabetes required insulin. Kaplan-Meier plots showed that 74/104 (71.1%) of high GADA titer required insulin compared to 45/108 (41.6%) of low GADA titer and to 86/412 (20.9%) of type 2 diabetes ($p<0.0001$ for both). BMI ≤ 25 kg/m² and positivity to IA-2IC and ZnT8 were also markers of faster progression ($p<0.0001$ for both). The proportion of LADA requiring insulin was significantly higher in the group of subjects treated also with sulfonylurea in the first year from diagnosis than in those treated with diet and/or insulin sensitizers ($p<0.001$). Multivariate analysis confirmed that presence of high GADA titer was a significant predictor of insulin ($p<0.0001$, OR=6.95).

Conclusion: High GADA titer, BMI ≤ 25 , ZnT8 and IA2IC positivity and sulfonylurea, in the first year from diagnosis, significantly increase the progression towards insulin requirement in LADA.

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Pre-existing insulin autoantibodies predict efficacy of otelixizumab in preserving residual beta cell function in recent-onset type 1 diabetes

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Background and aims: Immune interventions have shown partial and transient efficacy in preserving beta cell function in recent-onset type 1 diabetes. Future trials would benefit from biomarkers that predict therapeutic response, particularly when targeting subclinical disease. (Auto)antibodies against insulin (IA(A)), GAD (GADA), IA-2 (IA-2A) and ZnT8 (ZnT8A) were measured from this perspective during a randomized placebo-controlled anti-CD3-study (otelixizumab, GSK).

Materials and methods: Eighty recent-onset type 1 diabetic patients (n=40 otelixizumab, n=40 placebo) were included and levels of autoantibodies and stimulated C peptide release were measured at baseline (median duration of insulin treatment: 7 days) and every 6 months until 18 months after randomisation. IA(A), GADA, IA-2A and ZnT8A levels were determined by liquid-phase radio binding assays, C-peptide release was measured during hyperglycaemic glucose-clamp.

Results: At baseline and during follow-up, levels of GADA, IA-2A and ZnT8A were not significantly different between treatment arms. Compared to placebo treated patients, anti-CD3-treated participants experienced a less pronounced insulin-induced rise in IA(A), but only in initially IAA+ participants with relatively preserved beta cell function at baseline and concomitantly lower insulin needs. Univariate analysis identified IAA level at screening as well as C-peptide release at entry as predictors of anti-CD3 efficacy in terms of the preservation of beta cell function, but in multivariate analysis only IAA was retained ($p=0.026$).

Conclusion: Pre-existing insulin autoantibodies represent an independent marker for the therapeutic response of recent-onset type 1 patients to anti-CD3 treatment. Future immune intervention trials in (pre)type 1 diabetes should consider both presence of IAA and relatively preserved functional beta cell mass as inclusion criteria.

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Evaluation of DiaPep277 treatment in type 1 diabetes by integrated analysis

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Background and aims: Novel interventions evaluated in newly diagnosed type 1 diabetes (T1D) focus on preservation of beta cell function, glycemic control and insulin requirement. Primary study outcome is assessed by separate evaluation of each parameter, but the clinical benefit lies in the overall combined effect. Study DIA-AID 1 showed that treatment with DiaPep277* over 2 years resulted in a significantly better preservation of C-peptide and better glycemic control, in adult subjects newly diagnosed with T1D. The purpose of this analysis was to compare the effect of DiaPep277* to Placebo in populations of "Responder" and "Non-Responder" patients.

Materials and methods: The "Responder" profile was determined based on the following combined criteria: • Preservation of at least 80% of baseline stimulated and fasting C-peptide • HbA1c <8% • Insulin dose ≤ 0.5 IU/kg and no increase from baseline Data were analyzed using the SAS* version 9.1 (SAS Institute, Cary North Carolina). The Chi-square test was applied for analyzing the difference in proportion of responders and in other categorical parameters between the active and placebo groups. The two-sample T-test was applied for analyzing the difference in quantitative parameters between the active and placebo groups.

Results: Of the ITT population (N=457), 70% had full data (N=325) and could be profiled. In the DiaPep277* arm 53% met the criteria for "Responder." They presented at baseline with lower %HbA1c ($p<0.0001$) and also with

a higher age ($p < 0.0001$). In the Placebo arm, the 27% who met the criteria for “Responder” due to spontaneous good diabetes control, presented with significantly higher fasting and stimulated C-peptide levels ($p < 0.05$) and lower %HbA1c ($p < 0.0001$) at baseline. This increase of the “Responder” frequency from 27% in Placebo to 53% in DiaPep277* treated ($P < 0.0001$) is a major clinical benefit, as it represents a doubling in subjects whose overall deterioration is slowed by DiaPep277* treatment. Other baseline parameters showed no difference between “Responders” and “Non-Responders:” BMI, number of autoantibodies, gender, time from diagnosis, insulin dose and HLA.

Conclusion: This analysis identifies a quarter of subjects newly diagnosed with T1D as “Spontaneous Responders” whose presence in intervention studies adds to the variability of outcome. Still, on this background, half of adult patients treated for 2 years with DiaPep277* show a significant beneficial response when considering the integrated parameters of metabolic control.

Clinical Trial Registration Number: NCT00615264

PS 022 Beta cell ER stress and apoptosis

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Cross-interaction between C/EBPβ and AMPK determines the pancreatic beta cell mass

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Background and aims: Accumulation of C/EBPβ plays an important role in pancreatic β-cell failure caused by endoplasmic reticulum (ER) stress. Pancreatic β cell mass increases when gene modification or DPP-4 inhibitors are used to reduce expression of C/EBPβ accumulated in type 2 diabetes model mice or pancreatic β-cell specific C/EBPβ transgenic mice. We, therefore, aimed to elucidate the control mechanism of C/EBPβ expression.

Materials and methods: MIN6 cells were loaded with AICAR, metformin, and Extensin-4 to assess the relationship between AMPK activity and C/EBPβ expression levels. The effect of C/EBPβ phosphorylation on expression levels was also investigated. Further, vildagliptin and metformin were administered to pancreatic β cell specific C/EBPβ transgenic (TG) mice to investigate the relationship between C/EBPβ expression levels and AMPK activity in the pancreatic islets.

Results: In the pancreatic β-cells, ER stress causes enhanced expression and accumulation of C/EBPβ. The accumulated C/EBPβ was shown to inhibit AMPK activity by lowering the AMP/ATP ratio. C/EBPβ expression levels were, however, inhibited when AMPK was activated, and enhanced when AMPK was inactivated. In other words, AMPK activity and C/EBPβ expression levels were negatively correlated. Some of these effects are thought to involve T188 phosphorylation of C/EBPβ. In other words, the decreased AMPK was thought to increase C/EBPβ expression by raising the stability of C/EBPβ as a protein via enhanced T188 phosphorylation of C/EBPβ. In the pancreatic islets of the TG mice, increased C/EBPβ expression was accompanied by decreased AMPK activity. When the DPP-4 inhibitor vildagliptin was administered to the TG mice, the pancreatic islets exhibited improved AMPK activity accompanied by inhibited C/EBPβ expression. Moreover, with concomitant administration of vildagliptin and metformin, the pancreatic islets exhibited even greater activation of AMPK accompanied by even greater inhibition of C/EBPβ expression compared to vildagliptin alone. As a result, glucose tolerance was even further improved.

Conclusion: When pancreatic β cells are loaded with ER stress, enhanced C/EBPβ expression and decreased AMPK activity are thought to act synergistically to bring about pancreatic β cell failure. T188 phosphorylation of C/EBPβ also played an important in control of C/EBPβ expression by AMPK or ER stress. An increase in the pancreatic β cell mass and inhibition of C/EBPβ expression by vildagliptin was accompanied by activation of AMPK. Combining vildagliptin and metformin exhibited even greater AMPK activation accompanied by an increase in pancreatic β cell mass and inhibition of C/EBPβ expression, compared to vildagliptin alone. This suggests that decreased AMPK activity and enhanced C/EBPβ expression form a vicious cycle to bring about pancreatic β cell failure.

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Enhancing peroxiredoxin 4 expression improves insulin biosynthesis and glucose-induced insulin secretion in insulin-producing INS-1E cells

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Background and aims: Oxidative protein folding is crucial for the structural stability and proper function of most secretory proteins and polypeptide hormones such as insulin. This process takes place in the endoplasmic reticulum (ER) and is accomplished by protein disulfide isomerase (PDI) and ER oxidoreductin 1β (ERO-1β), generating stoichiometric amounts of hydrogen peroxide (H₂O₂) as byproduct. During insulin resistance in the pre-diabetic state, increased insulin biosynthesis can overwhelm the ER antioxidative and folding capacity, causing an imbalance in the ER redox homeostasis and

oxidative stress. Peroxiredoxin 4 (Prdx4), an ER-specific antioxidant peroxidase can utilize luminal H_2O_2 as driving force for reoxidizing PDI family members, thus efficiently contributing to disulfide bond formation. Therefore the aim of this study was to examine the functional significance of Prdx4 on β -cell function with emphasis on insulin content and secretion during stimulation with nutrient secretagogues.

Materials and methods: The ER-specific Prdx4 was specifically overexpressed in insulin-producing INS-1E cells. The Prdx4 overexpression was verified by Western blot, while its antioxidative effect was assessed by DCF in the presence of 5 mM dithiothreitol (DTT). Insulin content and secretion were determined by RIA and proinsulin transcription by qRT-PCR after stimulation with glucose (3, 10, 30 mM) and a combination of leucine (10 mM) plus glutamine (2 mM).

Results: Immunoblotting revealed that INS-1E cells stably transduced with a lentiviral construct carrying Prdx4 exhibited a significant increase in the Prdx4 expression compared to control cells. Exposure of control cells to DTT resulted in a significant generation of reactive oxygen species (ROS), whereas Prdx4 overexpression completely prevented the DTT-mediated ROS generation. Overexpression of Prdx4 led to an improved insulin secretion after stimulation with 10 and 30 mM glucose compared to control cells, while the basal insulin secretion at 3 mM glucose was not affected. An augmented insulin secretion could also be observed in Prdx4 overexpressing cells incubated with leucine plus glutamine. In addition, Prdx4 overexpressing cells exhibited an enhanced proinsulin mRNA transcription and insulin content when compared to control cells.

Conclusion: These data strongly suggest that enhancing ER-specific peroxiredoxin 4 expression in glucose-responsive insulin-secreting INS-1E cells significantly metabolized the DTT-mediated H_2O_2 generation within the ER and improved the glucose-induced insulin-secretion, which was accompanied by the enhancing proinsulin gene transcription and insulin content. This β cell beneficial effect was also observed upon stimulation with leucine, another nutrient insulin secretagogue, indicating that the effect is not restricted to glucose. Thus, Prdx4 improves the ER folding capacity and could contribute to the preservation of β cell function under conditions of high insulin requirement.

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Targeted overexpression of glycosylation-negative catalase mutations in the endoplasmic reticulum of insulin-producing cells

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Background and aims: In reaction to peripheral insulin resistance in type 2 diabetes mellitus, β -cells show initially an increased insulin secretion. Escalated insulin biosynthesis and secretion require an enhanced capacity for protein folding and disulfide bond formation, resulting in an increased production of H_2O_2 in the endoplasmic reticulum (ER). A direct quantification is not possible due to the oxidative environment in the ER and catalase overexpression for compensating an elevated H_2O_2 production failed due to N-glycosylation of the catalase protein. Therefore, a glycosylation-negative ER-targeted catalase variant, designed by mutagenesis, should be overexpressed to reduce the H_2O_2 concentration in the ER of insulin-secreting cells and to proof its potential impact on insulin secretion and ER stress.

Materials and methods: The two potential N-glycosylation sites of the human catalase protein were eliminated by PCR-mediated mutagenesis. The generated catalase variants were peroxisomally overexpressed to confirm that catalase enzyme activity was unaffected by the mutations, before the ER-specific expression was carried out in RINm5F and INS-1E cells. Catalase functionality in the ER and prevention of N-glycosylation was examined by enzyme activity measurement, incubation with H_2O_2 and Western blot analysis. Glucose-induced insulin secretion was quantified by RIA and typical ER stress markers were quantified by qRT-PCR analysis.

Results: Despite of the mutation of the N-glycosylation motives at asparagine-244 and -439, catalase activity was not affected by both single mutants (N244: 938 ± 114 , N439: 972 ± 101 , untransfected: 16 ± 4 U/ μ g protein). After overexpression of both variants in the ER only the ER-catalase-N244 showed enzyme activity and lack of glycosylation, whereas ER-catalase-N439 was still glycosylated and only a slightly elevated catalase activity could be detected (N244: 935 ± 51 , N439: 74 ± 10 U/ μ g protein). Quantification of catalase enzyme activity of the double mutant N244/439 after peroxisomal and ER-targeted overexpression revealed lower catalase activity in both compartments compared with N244 (peroxisome: 278 ± 17 , ER: 168 ± 12 U/ μ g protein). Measurement of glucose-induced insulin secretion and of ER stress-induced

genes showed no significant changes compared with untransfected control cells.

Conclusion: With the overexpression of ER-catalase-N244 a highly effective H_2O_2 inactivation within the ER could be achieved for the first time. Double mutation of both N-glycosylation motives N-244 and -439 was associated with partial loss of function, possibly due to the destruction of the three-dimensional enzyme structure. Since catalase has a high H_2O_2 inactivation capacity and is not involved in the protein folding process, the enzyme is an ideal tool for the investigation of insulin biosynthesis-associated oxidative ER stress, postulated for type 2 diabetes, or oxidative stress induced by misfolded protein aggregation in the ER. However, increased H_2O_2 inactivation through overexpression of ER-Catalase-N244 had no influence on insulin biosynthesis and the ER-stress response.

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ATF6 β regulates the *Wfs1* gene and has a cell survival role in the ER stress response in pancreatic beta cells

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Background and aims: Endoplasmic reticulum (ER) stress has been implicated in the development of pancreatic β -cell dysfunction and death resulting in type 2 diabetes. The Unfolded Protein Response (UPR) is the cellular system that responds to ER stress and consists of three main ER stress sensors, PERK, IRE1 and ATF6. Activating transcription factor 6 (ATF6) is an essential component of the UPR in cells undergoing ER stress that consists of two distinct genes, ATF6 α and ATF6 β . The ATF6 β isoform has been less studied and its role in the UPR in β -cells is unclear.

Materials and methods: Rodent islets and insulinoma cell lines were used to examine ATF6 β function. Microarray and qPCR validation was employed to identify ATF6 β target genes and siRNA knock-down and adenoviral overexpression was used to assess the role of ATF6 β in ER stress-induced apoptosis.

Results: ATF6 β mRNA and protein were detected in pancreatic β -cell lines and rodent and human islets and the protein was proteolyzed to the nuclear active form (ATF6 β p60) in response to pharmacological ER stress. Knockdown of ATF6 β using siRNA in INS-1 832/13 insulinoma cells did not affect mRNA induction of several major ER stress response genes in response to tunicamycin-induced ER stress, suggesting ATF6 β is not essential for the basic UPR. Expressing active ATF6 β p60 and ATF6 α p50 followed by microarray analysis revealed that ATF6 β and ATF6 α regulate similar UPR genes, including chaperones and ERAD components, although some genes such as *Wfs1* are ATF6 β -specific. Interestingly, knockdown of ATF6 β increased the susceptibility of INS-1 832/13 β -cells to apoptosis under both control and ER stress conditions, while overexpression of active ATF6 β p60 reduced apoptosis.

Conclusion: ATF6 β is not essential for induction of major ER stress response genes, but is required to maintain cell survival in β -cells undergoing chronic ER stress. The ATF6 β pro-survival role may relate to induction of genes that promote cell survival such as *Wfs1*.

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The novel translation initiation factor, eIF2A, is up-regulated by endoplasmic reticulum stress and protects beta cells from apoptosis

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Background and aims: Endoplasmic reticulum (ER) stress is an important mediator of pancreatic β cell loss in diabetes. Recent reports have associated inhibition of protein synthesis by the ER stress-induced unfolded protein response (UPR) with apoptosis. ER-stress associated translation reprogramming increase translation of stress-response mRNAs, but the signaling pathways that modulate this process in β cells are not well studied. Recently, the novel translation initiation factors eIF2A and eIF2D have been implicated in the translation of specific mRNAs under stress conditions in other systems. We investigated the role of eIF2A and eIF2D in the regulation of UPR gene expression and ER-stress-induced apoptosis in β cells.

Materials and methods: Thapsigargin (Tg, 1 μ mol/l), palmitate (PA, 0.5 mmol/l in BSA) were used to induce ER stress in primary mouse islets and mouse insulinoma cells (MIN6). Real-time RT-PCR, Western Blot and im-

munocytochemistry were used to assess gene expression. Apoptosis was assessed by flow cytometry of propidium iodide (PI) stained cells. Differences between means were considered statistically significant when $p < 0.05$.

Results: We found that eIF2A and eIF2D were expressed in mouse islets at both mRNA and protein levels. We compared eIF2A protein expression levels between different mouse tissues (heart, brain, liver, muscle, spleen, pancreatic islets) and found that they were highest in the pancreatic islets. eIF2D protein levels did not significantly differ between islets and other tissues. Fluorescent microscopy revealed that eIF2A and eIF2D were mainly localized in the cytoplasm of primary beta cells. Using MIN6 cells, we found gradual induction of eIF2A protein expression over the time course of Tg-induced ER stress, with the maximal fold increase of 1.78 ± 0.18 ($n = 4$) after 24 h, which coincided with activation of pro-apoptotic markers C/EBP homologous protein (CHOP, 137 ± 24 fold, $n = 4$) and cleaved caspase-3 (234 ± 11 fold, $n = 4$). We also observed a 1.66 ± 0.17 fold increase ($n = 4$) in eIF2D protein levels after 6 h of Tg-treatment that coincided with maximum Activating Transcription Factor-4 protein levels (8.7 ± 1.3 fold increase, $n = 4$). However, the effect was transient and after 24 h of Tg treatment, eIF2D protein levels were not different from the basal level. Treatment of MIN6 cells with PA for 24 h also significantly increased protein levels of eIF2A (2.1 ± 0.5 fold, $n = 3$) with no significant differences in eIF2D levels observed. Isolated mouse islets treated with either Tg or PA for 24 h showed elevated levels of eIF2A protein (by 1.35 ± 0.5 and 1.43 ± 0.2 fold respectively, $n = 3$) when compared to basal conditions, but not eIF2D. Importantly, we demonstrated that overexpression of recombinant eIF2A (10-fold) in MIN6 cells reduced thapsigargin-induced apoptosis by $51 \pm 15\%$ ($n = 3$). We found that decreased death of beta cells overexpressing eIF2A after 24 h of thapsigargin treatment was accompanied by decreased expression of UPR pro-apoptotic marker CHOP by $45 \pm 13\%$ ($n = 3$).

Conclusion: We conclude that eIF2A and eIF2D are expressed and differentially regulated over the course of UPR in beta cells. Also we identified a novel protective role for eIF2A in the context of ER-stressed beta cells via the inhibition of CHOP. Thus, eIF2A may potentially serve as a new therapeutic target in diabetes.

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The balance between adaptive and apoptotic unfolded protein responses regulates beta cell death through JNK, XBP1 and CHOP

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Background and aims: The loss of β -cell mass due to increased apoptosis is critical in both type 1 and type 2 diabetes. Pro-inflammatory cytokines and saturated fatty acids are potential mediators of β -cell apoptosis, although the mechanisms are poorly understood. Endoplasmic reticulum (ER) stress is present in β -cells in both type 1 and type 2 diabetes. ER stress is resolved through adaptive actions of the unfolded protein response (UPR). However, β -cells are prone to failure of the adaptive UPR with consequent activation of pro-apoptotic UPR, but the mechanisms are unclear. Here, we investigated the role of key ER stress-responsive transcription factors, XBP1 and CHOP, in the UPR induced by cytokines (IL-1 β , TNF- α and IFN- γ) or the saturated fatty acid, palmitate. We also examined the influence of Jun N-terminal kinase (JNK) activity in the UPR and the significance of these responses in β -cell survival.

Materials and methods: MIN6 β -cells and mouse islets were exposed for 24–48 h to the combination of pro-inflammatory cytokines, IL-1 β (100 U/ml), TNF- α (100 U/ml), IFN- γ (250 U/ml) or the saturated fatty acid, palmitate (0.4 mM coupled to 0.92% BSA). siRNA was used to silence XBP1 and CHOP in MIN6 cells. IRE1/XBP1 inhibitor (4 μ 8c - 30 μ M) was used in mouse islets. SP600125 (20 μ M) was used to inhibit JNK activity. β -cell death was measured using a cell death detection ELISA. Gene and protein expression changes were assessed by real-time PCR and Western blot.

Results: In MIN6 cells, exposure to cytokines or palmitate resulted in increased cell death and ER stress, which featured marked increases in pro-apoptotic UPR markers, CHOP, *Atf3* and *Trib3*. siRNA-mediated inhibition of XBP1 reduced the expression of adaptive UPR markers, including ER chaperones and foldases (*BiP*, *Grp94*, *Fkbp11*, *Erp72*, *Edem1*) and potentiated cytokine- and palmitate-induced apoptosis ($p < 0.001$). The enhancement of apoptosis was prevented by concomitant siRNA-mediated reduction of CHOP ($p < 0.001$). IRE1/XBP1 inhibition by 4 μ 8c also increased cytokine- and palmitate-induced apoptosis in mouse islets. These findings suggest that XBP1-mediated maintenance of the adaptive UPR inhibits CHOP-dependent

apoptosis following chronic cytokine and palmitate exposure in β -cells. Cytokine exposure also increased levels of phosphorylated JNK. JNK inhibition by SP600125 prevented the inhibitory effects of cytokines on XBP1 and adaptive UPR gene expression, lowered pro-apoptotic UPR gene expression, and protected β -cells from cytokine-induced cell death ($p < 0.001$).

Conclusion: These data suggest that JNK activity regulates the balance of the UPR in β -cells under conditions of ER stress; JNK activation inhibits XBP1-mediated UPR adaptation and promotes apoptotic UPR in β -cells. The differential regulation of adaptive and apoptotic UPR by JNK activation may provide a mechanism for the propensity of β -cells to apoptosis rather than ER stress adaptation in type 1 and type 2 diabetes.

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Chronic ethanol consumption inhibits Glucokinase transcriptional activity through ATF3/PDX-1/HDAC1 axis and triggers metabolic syndrome in vivo

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Background and aims: Chronic ethanol consumption induces pancreatic β -cell dysfunction through glucokinase (GCK) nitration and downregulation, leading to impaired glucose tolerance and insulin resistance; but the underlying mechanism remain largely unknown.

Materials and methods: To this, the 6-week male C57BL/6J mice were fed with 5% ethanol-containing liquid diet for 8 weeks and examined the effects of in vivo ATF3 silencing on GCK downregulation and metabolic alterations in ethanol-fed mice.

Results: Here, we demonstrate that GCK gene expression and promoter activity in pancreatic β -cells were suppressed by chronic ethanol exposure *in vivo* and *in vitro*; whereas expression of activating transcription factor 3 (ATF3) and its binding to the putative ATF/CREB site on GCK promoter were upregulated. Furthermore, *in vitro* ethanol-induced ATF3 inhibited the positive effect of PDX-1 on GCK transcriptional regulation, enhanced recruitment of HDAC1/2 and histone H3 deacetylation, and subsequently augmented the interaction of HDAC1/PDX-1 on the GCK promoter, which were diminished by ATF3 siRNA. Corroboratory, *in vivo* ATF3-silencing reversed ethanol-mediated GCK downregulation and β -cell dysfunction, followed by the amelioration of impaired glucose tolerance and insulin resistance. ATF3 association with T2D with alcohol consumption was supported by the identification of a novel non-coding variant, rs6670133, in the ATF3 gene after adjusting for age, sex, and alcohol consumption in Korean T2D population ($P = 0.041$).

Conclusion: We identified ethanol-induced ATF3 fosters β -cell dysfunction via GCK downregulation and that its loss ameliorates metabolic syndromes and could be a potential therapeutic target in treating T2D.

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Antiapoptotic HSPB1 mediates prolactin-induced cytoprotective effects on beta cells

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Background and aims: Transplantation of isolated pancreatic islets is an alternative for treatment of type 1 Diabetes; which is limited by the shortage of organ donors. Ex-vivo expansion of islet cell cultures appears as an attractive strategy; however, the islet fate in culture is determined, at least in part, by the balance between pro- and anti-apoptotic mediators. We have shown that recombinant human prolactin (rhPRL) inhibits beta-cell apoptosis. Moreover, we have recently reported PRL-induced up-regulation of the anti-apoptotic HSPB1 in human islets. Since the function of HSPB1 in beta-cells has not been directly studied, we set out to explore the role of HSPB1 in prolactin-induced beta-cell cytoprotection.

Materials and methods: We used primary cultures of human pancreatic islets and parental and HSPB1 knocked-down Min6 cells. Apoptosis was evaluated by DNA fragmentation and quantified by flow cytometry. Protein levels

of the Bcl-2 gene family members, caspase-9, HSTF1, P38, pP38, STAT-1 and pSTAT-1 were assessed by Western blotting. Caspase 3 and 8 activities were studied by fluorimetric assays.

Results: Our data showed that upon cytokines and rhPRL treatment, the proportion of fragmented nuclei was increased in HSPB1 silenced cells ($p<0.05$) when compared to control cells. In addition, the inhibition of cytokine-induced caspase-3 and caspase-8 activities as well as Bcl-2/Bax ratio and caspase-9 protein levels mediated by rhPRL in wild type cells was significantly reverted in knocked-down cells ($p<0.05$). Moreover, the kinetics of HSPB1 and HSTF1 expression levels were studied in primary cultures of human pancreatic islets which were serum starved and treated with rhPRL for short periods of time (from 10 min to 2h). The results showed that while HSTF1 presented a significant increase ($p<0.01$) in protein expression level after 10 min of rhPRL treatment, HSPB1 reached its maximum expression level upon 2h of hormonal treatment. Additionally, a significant ($p<0.05$) increase in STAT1 phosphorylation levels was detected after 10 min of rhPRL treatment reaching the highest peak upon 30 min ($p<0.001$) of hormonal treatment.

Conclusion: Therefore, we demonstrated a key role for HSPB1 in rhPRL-induced cytoprotective effects, since the lack of this protein completely abolished the beneficial effects induced by PRL on beta-cell death. Moreover, we provided for the first time, evidence for the co-regulation of HSPB1 and HSTF1 induced by rhPRL in human pancreatic cells which could be mediated by activated STAT1. Collectively, our results point to a promising target which could lead to the mitigation of beta-cell death through the up-regulation of an endogenous protective pathway which is not dependent on the modulation of the immune system.

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Modulation of glutamate signalling in human islets of Langerhans under hyperglycaemia

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Background and aims: Increasing evidence suggests that the excitatory neurotransmitter L-glutamate functions as a paracrine/autocrine signal in human islet of Langerhans. L-glutamate is released by α -cells together with glucagon and modulates hormone secretion by acting on specific glutamate receptors. When present at elevated concentrations, it may induce beta-cell death through oxidative stress. Its interstitial concentration is regulated by glutamate transporters of the SLC1A family which are expressed on the plasma membrane of endocrine cells. Their functional activity is essential for islet function as shown by the fact that their pharmacological inhibition increases glutamate concentration in the islets and causes beta-cell death. Aim of this study was to verify whether chronic hyperglycaemia may modulate the glutamate signalling system in human islets of Langerhans.

Materials and methods: Human islets were incubated under chronic (3 days) hyperglycaemia (16.7 mmol/l glucose) or normoglycemia (5.5 mmol/l glucose), and the expression and function of plasma membrane glutamate transporters and intracellular signalling proteins were studied by quantitative PCR analysis, western blotting, immunofluorescence, [3H]-Glutamate uptake and Ca^{2+} -imaging experiments.

Results: Quantitative PCR analysis revealed a $40\pm3\%$ reduction in the total ASCT2/SLC1A5 expression after incubation in chronic hyperglycaemia. No changes in the total GLT1/SLC1A2 mRNA and protein expression in human islets were detected. Immunofluorescence experiments performed on human islets exposed to hyperglycaemia revealed GLT1 relocalization into intracellular vesicular compartments of beta-cells. Because of this relocalization, the GLT1-mediated surface activity measured by [3H]D-glutamate uptake was inhibited by $31\pm5\%$ relative to normoglycemic conditions ($p<0.05$; $n=4$ in triplicate). Chronic hyperglycaemia induced a downregulation of the PI3K/Akt pathway in human beta-cells, suggesting a possible involvement of this pathway in the modulation of GLT-1 trafficking ($35\pm3\%$ downregulation of P-Akt expression, $n=5$ islet preparations). In line with this possibility, PI3K inhibition with 100 μM LY293 caused the GLT1 relocalization in intracellular compartments, and a $75\pm8\%$ downregulation of its activity, relative to control conditions ($p<0.001$; $n=2$ different islet isolations, in quadruplicate).

Chronic treatment with 10 μM ceftriaxone, a drug capable to upregulate GLT1 expression, significantly prevented hyperglycaemia-induced apoptosis in human islets ($65\pm12\%$ reduction of apoptosis. $P<0.001$; $n=2$ preparations, in quadruplicate).

Conclusion: Our data indicate that glutamate signalling in human islet is altered in hyperglycaemia and this may further contribute to beta-cell death. Targeting glutamate signalling system components may be a promising approach to prevent beta-cell death and to control glucose homeostasis in diabetes.

PS 023 Mechanisms of lipotoxicity

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Inhibition of *de novo* lipogenesis by lysosomal acid lipase in beta cells helps counter-regulate insulin secretion

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Background and aims: Endogenous lipolysis is hypothesised to contribute to the amplification phase of glucose-stimulated insulin secretion (GSIS) in pancreatic β -cells, mediated acutely by neutral lipases. Recent work shows that lysosomal acid lipase (LAL), an acidic lipase responsible for the breakdown of lipid delivered to the lysosome, is a negative regulator of GSIS. However, the exact mechanism of action of LAL and its role in whole body glucose homeostasis are unclear. Our current aims were to: (1) investigate glucose homeostasis in global LAL knock-out (KO) mice and (2) further characterise its mechanism of action.

Materials and methods: Wild-type (WT) and LAL KO mice were subjected to intraperitoneal glucose-tolerance tests (i.p. GTT). GSIS was measured by insulin RIA in islets isolated from WT and LAL KO mice. After 24 h LAL inhibition with Lalstat (5 μ mol/l) in MIN6 cells, gene expression was analysed by RT-PCR and lipid mass was assessed using mass spectrometry.

Results: LAL KO mice had significantly lower glucose excursions during an i.p.GTT compared to WT mice (AUC mean \pm SEM: WT 1514.3 \pm 104.8 n=10; LAL KO 1087.0 \pm 95.2 n=7; $P<0.05$ two-way ANOVA). This was most notable 30 min after the glucose bolus, and coincided with significantly higher insulin levels throughout the i.p.GTT (insulin AUC mean \pm SEM: WT 18.6 \pm 0.9 n=10; LAL KO 44.3 \pm 4.3 n=7; $P<0.001$ two-way ANOVA). To delineate a β -cell specific effect of LAL deletion, islets were assessed for GSIS *ex vivo*. Islets from LAL KO mice had increased GSIS at 20mmol/l glucose compared to WT islets (pg insulin/islet/h \pm SEM, n=5: WT 516.3 \pm 69.1; LAL KO 872.5 \pm 108.8; $P<0.05$ paired t-test). Together these data confirm that LAL has a physiological role in β -cell function and acts to inhibit insulin secretion *in vivo*. In mechanistic studies we determined that LAL inhibition in MIN6 cells enhanced expression of genes associated with *de novo* lipogenesis (Dgat1, Gpm1), as well as the lipase Lipe. The cholesterol transporter Abca1 was significantly downregulated. These data indicate that inhibition of LAL upregulates *de novo* lipogenesis, which acts to increase lipid signals that enhance GSIS. Consistent with this, mass spectrometry revealed that disaturated diacylglycerol mass was increased 3-fold after acute 20mmol/l glucose stimulation (n=4, $P<0.05$ unpaired t-test), and with LAL inhibition this was further potentiated 1.5-fold (n=4, $P<0.01$ paired t-test).

Conclusion: Our data further elaborate the importance of neutral lipid turnover in β -cell function, highlighting the key role of LAL in whole body glucose homeostasis and insulin secretion. In addition to its known role in regulating lipophagy, our data suggests that LAL mediated lipid breakdown also inhibits insulin secretion by downregulating *de novo* lipogenesis. It is likely that LAL plays a key role in limiting insulin secretion during starvation. *Supported by: NHMRC PhD scholarship and NHMRC project grants*

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Involvement of actin cytoskeleton in free fatty acid-induced beta cell death

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Background and aims: The role of actin cytoskeleton in regulation of pancreatic beta-cell survival has not been investigated. In this study, actin cytoskeleton remodeling was examined in the context of beta-cell apoptosis induced by free fatty acids (FFA).

Materials and methods: Studies were carried out in human pancreatic insulin-releasing 1.1B4 cells treated with 0.2 mM palmitate up to 24 h with or without pretreatment with 0.01 μ M cytochalasin D (CD) for 2 h or 25 nM jasplakinolide for 2 h. Actin cytoskeleton was visualized by immunofluorescence techniques. Expression and activation levels of the proteins under investigation were evaluated by immunoblotting techniques. Cell apoptosis was detected by measurements of cytosolic release of oligosomes (ELISA assay).

Results: Exposure of human 1.1B4 pancreatic beta-cells to 0.2 mM palmitate resulted in a 10- to 14-fold increase in cell apoptosis ($P<0.05$). The organiza-

tion of actin filaments was then examined in 1.1B4 beta-cells following exposure to palmitate. Palmitate induced a typical peripheral distribution of actin filaments consistent with their mechanical supporting function for shrinking of apoptotic cells. Treatment of beta-cells with cytochalasin D (CD) (0.01 μ M, 2 h) led to collapse of the filamentous actin structures and a more rounded cell shape, and inhibited the effect of palmitate on apoptosis ($P<0.05$). To further investigate the role of actin cytoskeleton in palmitate-induced apoptosis, 1.1B4 beta-cells were treated with jasplakinolide, a potent inducer of actin polymerization. Jasplakinolide (25 nM, 2 h) led to the formation of relatively fine stress fibers and large aggregations of actin filaments, and enhanced palmitate-induced apoptosis ($P<0.05$). In addition, palmitate-induced phosphorylation of the pro-apoptotic stress kinases JNK and p38 MAPK was reduced (by 45% and 90%) or increased (by 47% and 35%) after treatment with CD or jasplakinolide, respectively ($P<0.05$). Finally, palmitate induced a decrease in Akt phosphorylation, and this was prevented by CD ($P<0.05$); preincubation of beta-cells with the PI 3-kinase inhibitor LY294002 abrogated the ability of CD to restore Akt phosphorylation and to inhibit palmitate-induced apoptosis ($P<0.05$).

Conclusion: In conclusion, disruption and stabilization of actin cytoskeleton inhibit and enhance, respectively, FFA-induced beta-cell death. The essential role of actin cytoskeleton in FFA-induced apoptosis is coupled with activation of the pro-apoptotic JNK and p38 MAPK and inhibition of the anti-apoptotic Akt kinase.

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Gluco-lipotoxicity inhibits ceramide transport between endoplasmic reticulum and Golgi apparatus in pancreatic beta cells

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Background and aims: In type 2 diabetes (TD2) the chronic adverse effects of fatty acids (FA) on beta cell function and viability have been correlated to hyperglycaemia. Gluco-lipotoxicity refers to the combined, deleterious effects of elevated glucose and fatty acid levels on pancreatic beta-cell function and survival. Gluco-lipotoxicity caused beta-cell apoptosis and may thus contribute to progressive beta-cell loss in TD2. The molecular pathways and regulators involved in the detrimental effects of chronic exposure to FA, in particular palmitate, include the *de novo* synthesis of ceramide (Cer) in the endoplasmic reticulum (ER). However, increasing Cer levels in ER can also be due to a reduction of Cer utilization for complex sphingolipids synthesis. Therefore, we studied the effect of gluco-lipotoxicity on Cer metabolism in beta cells and its impact on beta-cell apoptosis.

Materials and methods: INS-1 cells were cultured with 0.4 mM palmitate and 5 or 30 mM of glucose. INS-1 cell apoptosis was determined by caspase 3/7 activity assay. Sphingolipid metabolites were analyzed by liquid chromatography-mass spectrometry. Metabolism of Cer was measured in INS-1 beta cells with [3H]sphingosine, a precursor of sphingolipid biosynthesis. Transport of C5-Bodipy-ceramides between ER and Golgi apparatus was analysed by fluorescence confocal microscopy. Down-regulation of Cer transporter CERT was made by specific siRNA.

Results: Both nutrients taken separately did not induce INS-1 cell death, whereas the combined treatment with palmitate and glucose resulted in an extensive beta cell apoptosis and this was associated to a significant increase of Cer levels. The presence of fumonisin-B1, an inhibitor of Cer biosynthesis, partially reversed the apoptosis induced by the combined treatment with palmitate and glucose. Metabolic studies using [3H]sphingosine as precursor of sphingolipid biosynthesis show that treatment with palmitate results in a small but significant increase of [3H]Cer associated to a decrease of [3H] sphingomyelin (SM) at 5 mM glucose. Inhibition of Cer utilization for SM biosynthesis was significantly potentiated by the presence of 30 mM glucose. Lipidomic analysis showed that gluco-lipotoxicity inhibited biosynthesis of complex sphingolipids. Fluorescence microscopy studies using C5-Bodipy-Cer show that at high gluco-lipotoxicity reduces the fluorescence accumulation in the perinuclear region representative of the Golgi apparatus. Interestingly, this was associated with an inhibition of the Cer transporter CERT function. Inhibition of CERT was mediated by a decrease of its expression and an increase of its phosphorylation status. Finally, selective silencing of CERT expression increased INS-1 cell apoptosis induced by palmitate.

Conclusion: Altogether these data suggest that gluco-lipotoxicity increased Cer accumulation in the ER through also through a decreased utilization of

newly synthesized Cer for SM biosynthesis. Moreover, these results support a role of Cer transport between ER and Golgi apparatus in the regulation of beta cell death induced by gluco-lipototoxicity.

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Palmitate and glucose induce autophagy in INS(832/13) cells

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Background and aims: Autophagy is an important biological process by which proteins and organelles are sequestered in autophagosomal membrane vesicles and delivered to the lysosome for degradation. Eukaryotic cells can degrade long-lived proteins and intracellular organelles through macroautophagy, for instance during periods of an increased energy demand. In addition, autophagy may function as a first line cellular defense to protect cells against various stressors and pathogens. The ATG genes, are crucial in regulating the formation of autophagy membranes, and dysfunctional regulation/expression of these may be involved in disease processes. The role of autophagy and/or regulation of ATG genes in type 2 diabetes are incompletely understood, although some evidence points to the involvement of autophagy in this disease. Therefore, the aim of this study was to investigate the exact cellular stressors that induce autophagy and ATG gene expression in beta cells in-vitro.

Materials and methods: The INS(832/13) beta cell line was cultured in combination with 30mM glucose, 0.5mM palmitate, or a combination of both, cytokines, LPS and tamoxifen (as a positive control) and untreated cells as a negative control. Incubations were performed for 6, 12, 24, and 48 hours to investigate the degree of autophagy visualized by fluorescent LC3B staining and confocal microscopy. Quantitative (Q)-PCR for ATG genes was performed in cells treated under each condition and insulin secretion analysis were performed following standard protocols.

Results: LC3B expression was used as the classical readout for autophagy. A preliminary time-course experiment evaluating INS(832/13) cells after 6, 12, 24, and 48 hours exposure to 30mM glucose and 0.5mM palmitate and a combination of both (glucolipototoxicity) indicated that autophagy response was maximal between 12 and 24 hours, and more prominent in cells treated with palmitate alone as compared to glucose treatment alone. The combination of both glucose and palmitate did not induce more autophagy than palmitate treatment, which was evaluated by counting the LC3B positive dots in each sample. The LC3B dot-like structures appear in the cytoplasm and perinuclear region and were more evident in all treated groups when compared to non-treated cells. An increase in expression was observed for ATG5, and ATG12 after 24 h incubation with palmitate. Insulin secretion was measured in cells treated in these conditions and preliminary data suggest a decreased in response to treatment with both palmitate and glucose. This indicates that the autophagic response induced by glucolipototoxicity inhibits insulin release, perhaps by mechanism relating to changes in autophagic response.

Conclusion: Our findings in vitro in INS(832/13) beta cells indicate that increased palmitate levels represent a powerful activator for the autophagy process which is coupled to an increase of specific ATG genes, necessary for the formation of the autophagosome. These changes are accompanied by a reduction in glucose stimulated insulin release.

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L-type voltage-gated calcium channels mediate sensitivity to glucolipototoxicity in beta cells via activation of nuclear receptors Nr4a and inhibition of autophagy

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Background and aims: Glucolipototoxicity is considered a major factor driving the gradual demise of pancreatic beta-cells mass in type 2-diabetes. Counteracting this, autophagy allows cells to survive under adverse conditions. Recent reports underscore the protective role of autophagy in animal models exposed to free-fatty acids and high concentrations of glucose. However, little is known about the involvement of autophagy in diabetes progression and its regulation in pancreatic B-cells is poorly understood. Under high glucose and palmitate conditions voltage-gated calcium channels (VGCC) become activated and in turn stimulate extracellular calcium-dependent gene expres-

sion. In this study we investigated which VGCC-activated genes are essential for autophagy regulation and whether deliberate tuning of expression of these genes under glucolipototoxic conditions could improve beta-cell viability through the activation of autophagy.

Materials and methods: Genes activated by VGCC were revealed by use of affymetrix microarray performed on INS-1 cells after stimulation with 70mM potassium chloride. Amongst them autophagy related genes were identified based on the literature studies. Expression of these genes was measured by quantitative PCR and western blot. Their relation to the type of VGCC was obtained owing to specific VGCC inhibitors. Autophagy was assessed by use of western blot where the ratio of LC3B II over I form was calculated, and as well as by use of confocal microscopy on INS-1 cells wherein the number of LC3B-GFP puncta was counted. Apoptosis was measured by use of both flow cytometry (the number of AnnexinV and ViaProbe positive cells) and ELISA assay measuring enrichment of nucleosomes in cytosol.

Results: Affymetrix microarray revealed that activation of VGCC with 70mM potassium chloride upregulated autophagy related nuclear receptors Nr4a: NUR77 and NOR-1. Quantitative PCR showed 10-times increase of NUR77 and NOR-1 genes under high potassium conditions. At the same time activation of VGCC inhibited autophagy, which was indicated by 20% decrease in LC3B II/I ratio and 50% decrease in the number of LC3B-GFP puncta in beta-cells. This coincided with 50% reduction of AMPK phosphorylation - the main autophagy activator. Inhibition of VGCC-activated NUR77 and NOR-1 expression by isradipine or downregulation of both genes with siRNA restored AMPK activity and ablated inhibitory effect of high potassium on autophagy. 25 mM glucose with 0.5 mM palmitate inhibited autophagy which was indicated by 70% decrease in LC3B-GFP puncta and increased 10 times the number of AnnexinV and ViaProbe positive cells as compared to low glucose conditions. Isradipine or autophagy inducing rapamycin reduced apoptosis by 20% under glucolipototoxic conditions. Finally, cells depleted of both NUR77 and NOR-1 gained 25% improvement in cell viability at the presence of high glucose and palmitate.

Conclusion: Our study shows that inhibition of autophagy by L-type VGCC makes beta-cells more vulnerable to apoptosis under glucolipototoxic conditions. Beneficial for cell viability activation of autophagy can be obtained by inhibition of L-type calcium currents as it prevents induction of anti-autophagic - NUR77 and NOR-1.

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Lipotoxicity alters the genome-wide epigenetic pattern in human pancreatic islets

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Background and aims: Circulating levels of free fatty acids are often increased in subjects with type 2 diabetes (T2D). Long-term exposure to lipids has harmful effects on islet function and insulin secretion. Epigenetic modifications such as DNA methylation may contribute to T2D. However, there is limited information on whether fatty acids alter the epigenetic pattern in human pancreatic islets. Our aim was therefore to analyse the genome-wide DNA methylation pattern in human pancreatic islets exposed to palmitate for 48 hours and relate methylation to gene expression and insulin secretion in the islets.

Materials and methods: mRNA expression and DNA methylation were analysed genome-wide in human islets using microarrays.

Results: Palmitate treatment for 48 hours decreased glucose-stimulated insulin secretion but did not affect apoptosis in the human islets. We found 1860 genes with differential expression in palmitate-treated human islets. These include candidate genes for T2D such as GLIS3, HNF1B and SLC30A8. Additionally, palmitate altered the expression of genes in glycolysis/gluconeogenesis, pyruvate metabolism, fatty acid metabolism, glutathione metabolism in human islets. The global DNA methylation level and DNA methylation levels of CpG island shelves and shores, 5'UTR, 3'UTR and gene body regions were altered in human islets exposed to palmitate. Moreover, 290 genes with differential expression had a corresponding change in DNA methylation e.g. several candidate genes for T2D. Importantly, 67 of these genes were also associated with BMI and 37 were differentially expressed in islets from T2D patients.

Conclusion: We demonstrate that lipotoxicity gives rise to epigenetic modifications as well as transcriptional changes in human pancreatic islets. These changes may contribute to impaired insulin secretion and T2D.

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EPA and DHA protect pancreatic islets against palmitate toxicity

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Background and aims: In previous studies, we have shown that fish oil supplementation improve the antioxidant defense in pancreatic islets from healthy rats. To test whether these effects were due to ω -3 fatty acids present in high concentrations in the supplemented diet, we decided to test whether EPA and DHA have the same effects in vitro and protect pancreatic beta cells from palmitate toxicity.

Materials and methods: Pancreatic islets were obtained by collagenase digestion and cultured with RPMI 1640 medium containing 10mM glucose, 10% FBS, penicillin and streptomycin. After overnight culture, islets were divided in four groups: control (vehicle ethanol), ω -3 fatty acids (50 μ M EPA + 50 μ M DHA), 100 μ M Palmitate (P), Pw3 (50 μ M EPA + 50 μ M DHA + 100 μ M palmitate). Islet cell death, glucose stimulated insulin secretion (GSIS) and superoxide (ROS) content (cytosolic and mitochondrial) were analyzed after 48 h culture. Some of the experiments were performed using dispersed cells. Briefly, after 48 h culture, islets were dispersed into small cell clusters using trypsin and gentle pipetting in a Ca^{2+} -free medium. Cells were then analyzed by flow cytometry.

Results: 48h culture in the presence of P increased beta cell ROS production and apoptosis and impaired GSIS. Under these conditions, EPA+DHA triggered a parallel ~60–65 % reduction in ROS production and beta-cell apoptosis induced by P, and induced significant protection against the impairment in GSIS (~1,28ng insulin in P vs ~1,60ng insulin in Pw3, per islets/hour). The reduction in insulin secretion was not due to decreased insulin content. When dispersed cells were analyzed by flow cytometry, there was no difference in mitochondrial ROS production (mitosox probe). On the other hand, ROS production was increased with P and reduced to control levels when P cells were cultured in the presence of EPA and DHA, confirming the results obtained with whole islets using confocal microscopy. Apoptosis was also increased in P vs control and the addition of ω -3 is not significantly different from control.

Conclusion: Our results show that EPA+DHA protect pancreatic islets from alterations induced by palmitate, i.e. increased superoxide production, decreased viability and beta cell function. The effects may result from decreased cytosolic superoxide production, which in turn can lead to increased viability and beta cell function. As we previously reported, in vivo ω -3 supplementation decrease protein levels of NAD(P)H oxidase subunits and consequently superoxide production in pancreatic islets. Considering that in vitro effects do not involve mitochondrial superoxide production, we can speculate that this protection might involve downregulation of NAD(P)H oxidase. However, further experiments are needed to elucidate the mechanisms involved in the protection of ω 3 fatty acids against palmitate toxicity.

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Different regulation of beta cell proliferation induced by short-term and long-term high-fat diet loading

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Background and aims: Evidence has mounted that insufficient adaptation of beta cell mass is symptomatic of type 2 diabetes. Because the adaptation mechanism of beta cell mass in response to insulin resistance is not fully understood, elucidating this mechanism is absolutely required for developing strategies of diabetes treatment. Previously, we demonstrated that insulin receptor substrate-2 (Irs2) is critically required for beta cell proliferation to occur in response to high-fat (HF) diet-induced insulin resistance. Recently, it was reported that beta cell proliferation began within the first 7 days of HF diet loading. However, it is unclear whether Irs2 is required for beta cell

proliferation induced by short-term HF diet loading. Here, we investigated the effect of short-term HF diet loading on the regulation of beta cell proliferation.

Materials and methods: Eight-week-old C57bl/6J mice were given free access to either standard chow (SC) or a HF diet. After 7 days on the above diets, we investigated body weight, blood glucose, visceral fat weight, liver weight and pancreatic weight in these mice. Also, insulin tolerance test and immunohistochemical analysis to assess beta cell proliferation and mass were performed. Furthermore, we evaluated the changes in expression levels of genes involved in beta cell proliferation and function in islets isolated from these mice.

Results: Body weight and fed blood glucose levels were significantly higher in the mice on the HF diet than those in the mice fed SC. Although visceral fat weight were significantly higher in the mice on the HF diet than those in the mice fed SC, there were no differences in liver weight or pancreatic weight between the two groups. The glucose-lowering effect of insulin in the mice on the HF diet was equivalent to that in the mice fed SC on day 6. Immunohistochemical analysis revealed that there was a significant increase in stimulated the BrdU incorporation rate in the mice on the HF diet in comparison with the mice fed SC on day 7, although there was no difference in the area of the beta cells relative to that of the whole pancreatic tissues between the two groups. Real-time quantitative PCR showed that Ki67 and Cyclin A2 mRNA were significantly increased in the mice on the HF diet in comparison with the mice fed SC. However, no increase in the expression levels of Irs2 or genes involved in beta cell function, such as pancreatic and duodenal homeobox-1, glucokinase, insulin-1, or insulin-2 were noted in the mice on the HF diet as compared with that in the mice fed SC.

Conclusion: Beta cell proliferation was induced by HF diet loading only for 7 days without up-regulation of Irs2. Our results suggest different regulation of beta cell proliferation induced by short-term and long-term high-fat diet loading.

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Metabolic and pancreatic effects of transplantation of mesenchymal stem cells in a model of insulin resistance and type 2 diabetes

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Background and aims: Type 2 Diabetes Mellitus (DM2) is associated with insulin resistance and dysfunction of pancreatic β cells. The regenerative cellular therapy, in particular with multi/pluripotent cells has been investigated as a potential therapeutic strategy for DM2. Among them, mesenchymal stem cells (MSCs) due to its immuno regulatory role are important therapeutic candidates. The purpose of this study was to investigate the effects of multiple infusions of MSCs on glucose homeostasis and morphometry of the pancreatic islets in high fat diet-induced diabetes in Swiss mice.

Materials and methods: Swiss mice were fed a standard diet or a high fat diet for eight weeks. The animals were then divided into 3 groups: non-diabetic group (fed a standard diet), untreated diabetic group and MSCs transplanted group. The transplanted mice received 4 intraperitoneal infusions of MSCs cells ($5-8 \times 10^6$ MSCs resuspended in buffer). Diabetic untreated animals received only buffer injection and non-diabetic group did not receive injections. Fasting plasma glucose (FPG) was determined weekly and glucose (GTT) and insulin (ITT) tolerance tests were performed at 1, 2, 3, and 4 months after the infusions of MSCs. Four months after infusion of the MSCs, the animals were decapitated and pancreas and serum were collected for analysis.

Results: The MSCs transplanted animals were classified as responder (FPG < 180mg/dL) or non-responder (FPG > 180mg/dL). According to this criterion, 72.2 % and 27.8 % of MSCs transplanted animals were classified as responders and non-responders, respectively. Fasting glycemia decreased significantly ($p < 0.05$) in responders mice seven weeks after the infusion of MSCs compared to the untreated diabetic group. Four months after MSCs, GTT and ITT areas under the curve (AUC) decreased significantly ($p < 0.05$) in responders mice compared to untreated diabetic mice. Serum insulin concentration was significantly higher in untreated diabetic animals than in non-diabetic animals ($p < 0.05$) and was not different in responders and non-responders transplanted mice. Total islet area and volume of β cells were not different among the groups. However, the volume of α cells was significantly ($p < 0.05$) lower in untreated diabetic animals and responders mice compared to non-diabetic and non-responders mice, respectively. Apoptosis of islet cells was significantly ($p < 0.05$) bigger in untreated diabetic animals

than in non-diabetic mice, and significantly lower in responders mice than in untreated diabetic and non-responders animals. Islet cell proliferation was significantly ($p < 0.05$) lower in untreated diabetic animals than in non-diabetic mice. However, islet cell proliferation was not different in transplanted animals compared to non-diabetic and untreated diabetic animals. There was a positive correlation between apoptosis of islet cells and fasting glycemia ($r = 0.56$; $p = 0.002$) and AUC of GTT ($r = 0.59$; $p = 0.001$) and ITT ($r = 0.42$; $p = 0.03$), and a negative correlation of proliferation of islet cells and fasting glycemia ($r = 0.39$; $p = 0.03$).

Conclusion: The results indicate that multiple infusions of MSCs decrease glucose intolerance and apoptosis in pancreatic islets and increase insulin sensitivity in high fat diet-induced diabetic mice.

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Absence of CXCL10 prolongs islet graft survival in an autoimmune transplantation setting

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Background and aims: Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-producing beta-cells in the pancreas. The chemokine CXCL10 seems to play an important role in the recruitment of autoaggressive lymphocytes to the islets of Langerhans. Since transplantation of isolated islets is a promising therapy of T1D, but has been hampered by the fact that islet grafts get rejected even in presence of an immunosuppressive regimen, we intended to investigate a possible influence of CXCL10 on the islet rejection process.

Materials and methods: We used three different transplantation settings, including syngeneic, allogeneic and autoimmune transplantations. For the syngeneic and allogeneic transplantation we used streptozotocin-treated diabetic recipients (C57Bl/6 or FVB background). In order to investigate the autoimmune rejection we used diabetic RIP-LCMV-GP mice as recipients. The RIP-LCMV-GP mice express the glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) under control of the rat insulin promoter (RIP) specifically in the beta-cell of the pancreatic islets of Langerhans. One of the advantages of the RIP-LCMV-GP model is the uses of a well-defined target autoantigen which allows a precise observation and quantification of the destructive immune response resulting in the islet graft rejection. Pancreatic islets were isolated from either C57Bl/6 and CXCL10-deficient mice (syngeneic and allogeneic settings) or RIP-LCMV-GP and RIP-LCMV-GP x CXCL10-deficient mice (autoimmune setting) and were transplanted under the left kidney capsule of diabetic recipient mice.

Results: CXCL10 was expressed by islet grafts early after transplantation. However, no significant differences in the survival of wildtype or CXCL10-deficient islets have been observed in the syngeneic (C57Bl/6 islets into C57Bl/6 recipients) and allogeneic (C57Bl/6 islets into FVB recipients) settings. In contrast, autoimmune (RIP-LCMV-GP islets into RIP-LCMV-GP mice) transplantations demonstrated a marked prolongation of islet graft survival in the absence of CXCL10 in the transplanted islets. Insulin production was intact for more than 100 days post-transplantation in non-rejected islet graft.

Conclusion: Consequently, CXCL10 might play a role in islet graft rejection in an autoimmune transplantation setting, and might be a potential therapeutic target to prolong islet graft survival.

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Improved revascularization of islet graft by angiogenic monocyte subpopulation derived from spheroid culture of bone marrow mononuclear cells

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Background and aims: Spheroid culture method is an effective strategy for the ex vivo expansion of autologous therapeutic cell population. We investigated if co-transplantation of spheroids (BM-spheroid) formed by 3D culture of bone marrow-derived mononuclear cells (BM-MNCs) could improve the revascularization and organization of islet grafts.

Materials and methods: Angiogenic capacity of the spheres formed by spheroid culture of BM-MNCs (BM-spheres) for 3 to 5 days was assessed by in vitro and in vivo models. A syngeneic marginal mass renal subcapsular islet transplantation model was used to determine the post-transplant outcome of co-transplanted BM-spheres and islets. Using green fluorescent protein transgenic (GFP-Tg) mice, the role of BM-spheroid and donor/recipient endothelial cells in revascularization was assessed by immunohistochemistry.

Results: The main cellular component of BM-spheres were CXCR4⁺CD14⁺ myeloid cells. BM-sphere showed a superior angiogenic capacity over the fresh BM-MNCs and remnant non-spheroid BM-MNCs after spheroid culture. Co-transplantation with BM-spheres improved the outcome of syngeneic islet transplantation in terms of glucose tolerance, diabetes reversal

rate, plasma insulin levels, and fraction of endocrine cells and proliferating β -cells in graft immunohistochemistry. Co-transplantation with BM-spheres increased the vessel density derived from the donors and recipients.

Conclusion: Co-transplantation of islets and angiogenic myeloid cells derived from the BM-spheroid improved the outcome of marginal mass islet transplantation by facilitation of revascularization.

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First evaluation of a closed, continuous media renewal system for human islets of Langerhans

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Background and aims: Many clinical islet centres have adopted a short storage period for islets prior to transplantation. A storage period provides time for quality assessment of islets, recipient matching, transport of islets and patients to transplant centre and initiation of immunosuppressive protocols. There are, however, risks for loss of islet tissue and contamination during the storage period. To reduce contamination risks and improve handling and storage conditions, the present study presents the first evaluation data from of a new closed PReservation ISlet system (called PRISM) specifically developed for islet storage with regulated continuous media renewal.

Materials and methods: At the end of the isolation process, purified islets were split and stored either in single transfer packs for platelets (Fenwal, Sweden) as control or in the new V1.4 PRISM automate (Macopharma, France) for 4–5 days. Islets were both kept in CMRL-1066 supplemented with 10 mM HEPES, 10 mM nicotinamid, 2 mM L-glutamine, 50 μ g/mL gentamicin, 5 mM sodium pyruvate, 20 μ g/mL ciprofloxacin and 10% blood group-compatible human serum. After a first over night storage at 37°C, the temperature was lowered to 25°C for the rest of the study period. Culture media exchanges were either performed manually for islets in the platelet bags or automatically using the PRISM automate. Islet quality assessments were performed at day 1 and last day (4 or 5) and included glucose-stimulated insulin release, intracellular insulin content, ADP/ATP ratio, cytokine expression and recovery.

Results: No differences were found between islets kept in the PRISM automate compared to islets kept in single transfer packs for platelets regarding stimulated insulin release, intracellular insulin content, ADP/ATP ratio or expression of MCP-1, tissue factor, IL-6 or IL-8.

Conclusion: The closed, automatic media renewal PRISM technology seems to preserve functional integrity of clinical grade human islets kept in storage as well as standard clinical practice. It presents an attractive method for standardization and automation of islet storage.

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Feasibility of islet magnetic resonance imaging using ferumoxytol in intraportal islet transplantation

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Background and aims: There is a clinical need for an alternative labeling agent for magnetic resonance imaging (MRI) in cell therapy including islet transplantation, because superparamagnetic iron oxide (SPIO) has been withdrawn from the market. We aimed to evaluate the feasibility of islet magnetic resonance imaging using ferumoxytol, which is a clinically available ultrasmall superparamagnetic iron oxide (uSPIO) as an iron supplement drug.

Materials and methods: We compared islet function and viability of control islets and islets labeled with ferumoxytol. In vitro efficacy of ferumoxytol labeling was assessed with prussian blue stain, electron microscopy and ex vivo MRI of labeled islets. In vivo efficacy of labeling was assessed in both renal subcapsular and intraportal islet transplantation models.

Results: Labeling with ferumoxytol up to 800 μ g/mL did not compromise the viability and glucose-stimulated insulin secretion of labeled islets. Prussian blue stain of labeled islets showed internalized ferumoxytol particles. Ex vivo magnetic resonance imaging of islets labeled with ferumoxytol (up to 800 μ g/mL) for 48hr revealed visible hypointense spots representing labeled islets. In syngeneic renal subcapsular islet transplantation model, islet MRI at 14 days-post-transplantation (DPTs) showed visible hypointense spots representing islet graft. In islet MRI at 7 and 14 days after syngeneic intraportal

islet transplantation, there was a difference in the total area of hypointense spots between recipients with normoglycemia and hyperglycemia at 28 DPTs. **Conclusion:** Islet MRI using ferumoxytol was feasible in terms of in vitro and in vivo efficacy and safety. Labeling islet with ferumoxytol could be a useful option for estimation of islet mass in clinical islet transplantation.

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Outcomes for adults with type 1 diabetes referred with severe hypoglycaemia and/or referred for islet transplantation to a specialist hypoglycaemia service

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Background and aims: Severe hypoglycaemia (SH) and fear of hypoglycaemia remain major barriers to achieving good diabetes control. In adults with type 1 diabetes (T1D), structured education in flexible insulin therapy (e.g. DAFNE, Dose Adjustment For Normal Eating) reduces SH by 65%, and technology (insulin pump therapy and sensors) 4-fold. For persistent SH, despite optimised medical therapy, islet cell transplantation is indicated, reducing SH from 20.0 [7.0–50.0] to 0.3 [0.0–1.6] episodes per patient/year. We examined outcomes for patients referred to a diabetes centre with an islet transplant unit for islet transplantation and/or problematic hypoglycaemia.

Materials and methods: Retrospective case note audit of all people with T1D referred to islet transplant unit for islet transplantation and/or with problematic hypoglycaemia between 2009 and 2012 [n=82]. 45 met criteria for islet cell transplantation having >1 SH in previous year. Optimal outcome was defined as ≤ 1 severe hypoglycaemic episode over the past 12 months as documented at most recent visit.

Results: The cohort was 55.6% male, mean (\pm SD) age 44.8 (± 11.7) years, BMI 25.2 (± 3.6) kg/m² and duration of diabetes 28.1 (± 13.4) years. HbA1c at index visit was 8.5% (± 1.8), median [IQR] frequency of severe hypoglycaemia was 6.0 [2.0–21.5] per patient/year and 84.4% had impaired awareness of hypoglycaemia. 84.4% were referred from secondary diabetes services, 13.3% had completed DAFNE and 31.1% were using insulin pumps. Nine patients (20.0%) had initial assessment only (3 died, 2 did not attend follow up and 4 were referred back to local team with a new management plan). Follow up of the remaining 36 had a median duration of 28.5 [17.8–42.5] months, during which SH fell from 6.0 [2.0–24.0] to 0.0 [0.0–3.0] events per patient/year; $p < 0.0001$, without deterioration in HbA1c [8.3% to 8.1% [67.2mmol/mol vs. 65.0mmol/mol]; $p = 0.239$]. 26 out of 36 remained on medical therapy (11 had dose adjustment alone; 11 completed DAFNE; 10 converted to pump; 3 used sensors). 17 out of 26 achieved optimisation (13=0 SH and 4=1 SH) with a SH reduction from 2.0 [1.5–9.0] to 0.0 [0.0–0.5] episodes per patient/year; $p < 0.0001$, again without detrimental effect on HbA1c [8.1% to 7.7% [65mmol/mol vs. 60.7mmol/mol]; $p = 0.072$]. 9 out of 26 were not resolved but SH fell from 7.0 [4.8–40.5] to 4.0 [2.5–6.3] episodes per patient/year; $p = 0.058$. The remaining 10 patients were listed for transplantation. 80% had completed DAFNE, 100% converted to insulin pump therapy and 70% used sensors. There was a trend to higher baseline SH rate in the group needing transplantation [2.0 [1.5–9.0] (optimised) vs. 7.0 [4.8–40.5] (improved) vs. 8.0 [5.3–106.0] (transplantation); $p = 0.060$]. Overall, 47.2% of patients were resolved; 25.0% improved and 27.8% required transplantation.

Conclusion: The majority of patients presenting with problematic severe hypoglycaemia can be improved with appropriately supported non-transplant therapeutic options, around half achieving complete resolution. Despite optimised therapy, a third will require transplantation. Provision of expertise in hypoglycaemia management is essential to give people with T1D and SH appropriate options and focus limited transplant resources on the most needy.

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Meal tests to assess beta cell function and mass in islet-transplanted patients

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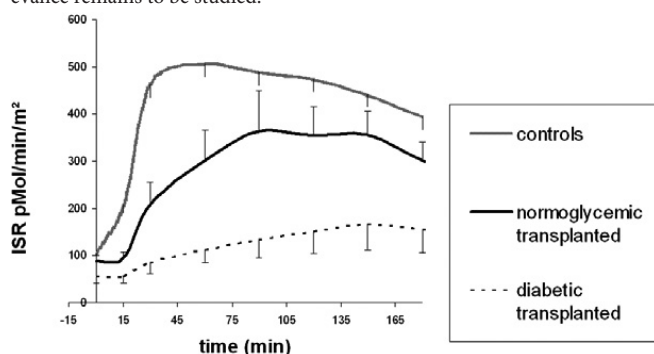
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Background and aims: Current model-derived calculations of insulin secretion based on C-peptide kinetics during a meal test allow to measure the two phases of insulin secretion. The second phase expressed as a β -cell glucose sensitivity (BCS) has been shown to be a strong predictor of diabetes progression, and recent studies also suggest that it is closely related to the size of pancreatic beta-cell mass. Since beta-cell mass is a critical factor for the outcome of pancreatic islet transplantation, we measured these parameters in transplanted patients in order to see whether they were related to glucose tolerance.

Materials and methods: We performed 13 standardized breakfast tests (76 g of carbohydrates) in 7 patients treated with islet transplantation (5 F/2 M, age 32–60 yr; weight: 51.3–72.8 kg, time after transplantation 3–44 months) and compared them to 103 non-diabetic controls (C). Calculation of insulin sensitivity (Caumo's oral minimal model) and insulin secretion rate (ISR) (Van Cauter's model), with calculation of beta-cell sensitivity to glucose (BCS) and insulin secretion parameters given by the classical models of Breda and of Mari were done with the results of the tests.

Results: According to fasting glycemia and glycemia at 2 hours, breakfast tests showed 6 normoglycemic profiles (NP) and 7 diabetic profiles (DP). NP patients had significant lower HbA_{1c} (5.9% VS 7.1%; $p < 0.001$) and were insulin-independent compared to DP who all resumed insulin. No differences were observed between groups regarding time after transplantation. Comparison of NP and DP with C showed that total ISR was higher in C than in the two groups of transplanted patients: 110.98 ± 4.11 pMol/m² in C vs 61.17 ± 9.75 ($p < 0.005$) in NP and 26.88 ± 7.78 in DP ($p < 0.02$ compared to NP). Maximal insulin secretion exhibited the same trend: C: 777.27 ± 31.51 pMol/min/m² > NP 421.24 ± 72.76 > DP 181.80 ± 52.25 (C vs NP $p < 0.01$; NP vs DP $p < 0.02$). The most significant difference was found with beta-cell sensitivity C: 126.90 ± 5.94 pMol/min/mmol/m² > NP 61.53 ± 11.73 > DP 12.89 ± 3.72 (C vs NP $p < 0.005$; NP vs DP $p < 0.01$). First phase index calculated according to Breda's model was also lower in DP vs NP ($p < 0.001$). Insulin sensitivity did not differ among the 3 groups.

Conclusion: These results show that insulin secretion remains lower than normal in islet-transplanted patients if they have normal glucose tolerance, and is even lowered if they are still diabetic. BCS values are extremely low when islet-transplanted patients are diabetic and remain lower than normal (in the range observed in type-2 diabetics) when they have a normal glucose tolerance. This quantitative evaluation of insulin secretion is thus likely to be useful for the follow up of islet transplantation, even if its prognostic relevance remains to be studied.



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Actual 10 year pancreas transplant alone (PTA) results in a single centre experience

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Background and aims: PTA for the treatment of selected type 1 diabetic patients is receiving increasing attention. However, information on PTA long-term outcome is still limited. Aim of this study was to investigate the results of PTA performed in our center after 10 yrs of follow-up.

Materials and methods: From December 2000 to December 2003, 34 type 1 diabetic patients (age: 37 ± 9 yrs; 17 males/17 females; BMI: 23.5 ± 3.3 kg/m², duration of diabetes: 23.6 ± 10.1 yrs, insulin requirement: 47 ± 10 UI/day) underwent PTA with the portal-enteric drainage approach. Immunosuppression consisted of basiliximab and high dose steroids as induction, and mycophenolate mophetil, tacrolimus and low dose steroid as maintenance treatment. Actual patient and pancreas (insulin-independence) survival, biochemical parameters and indexes of cardiovascular status were analyzed at 10 yrs after transplantation.

Results: Ten-year patient survival was 97% (33/34 patients; 1 death with functioning graft occurred at 5 yr post-transplant due to stroke), and death censored pancreas survival was 63.6%. Failures were due to acute rejection (2 cases) and chronic rejection (10 cases). Patients with functioning grafts were normoglycemic in the absence of exogenous insulin therapy (fasting plasma glucose: 96 ± 19 vs 230 ± 108 mg/dl, HbA_{1c}: 5.8 ± 0.6 vs $8.3 \pm 1.8\%$, both $p < 0.001$ vs pre-transplantation values), with sustained endogenous insulin secretion (C-peptide levels: 2.8 ± 1.5 vs 0.1 ± 0.1 ng/ml, $p < 0.001$). Total cholesterol (157 ± 40 vs 193 ± 31 mg/dl, $p < 0.001$) and LDL cholesterol (95 ± 36 vs 128 ± 36 mg/dl, $p < 0.001$) were lower after PTA, with no significant changes in statin therapy. Blood pressure values resulted similar before as after transplantation (systolic: 126 ± 12 vs 125 ± 17 mmHg; diastolic: 74 ± 10 vs 78 ± 10 mmHg), with unchanged anti-hypertensive therapy. Estimated glomerular filtration rate (EPI-MDRD) decreased at a rate of 1.7 ± 2.1 ml/min/yr, and albumin excretion rate was lower after PTA (0.17 ± 0.24 vs 1.6 ± 2.9 g/24h, $p < 0.05$). Left ventricular ejection fraction increased after transplantation from 54.1 ± 4.3 to $58.9 \pm 2.7\%$ ($p < 0.01$).

Conclusion: In this single centre experience, PTA successfully and safely allowed long-term (10-yr) actual patient survival and insulin-independence, and associated with improvement of several biochemical and clinical parameters.

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Diabetic neuropathy in pancreas and kidney transplant recipients: follow-up after 8 years of normoglycaemia

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Background and aims: Very advanced forms of diabetic peripheral neuropathy are generally present in pancreas/kidney transplant candidates. We present long-term follow-up data on the effect of normoglycemia after a simultaneous pancreas/kidney transplantation (SPK) on epidermal nerve fibre (ENF) density and neurological function in type 1 diabetic (DM) recipients.

Materials and methods: Lower thigh 3-mm skin biopsies with ENF counts (indirect immunofluorescence method), autonomic function (AFT) and quantitative sensory testing (QST) and electrophysiological examinations were performed at time of and at 8 years post-SPK in 12 Type 1 DM patients (mean \pm SD age 44 ± 10 ; pre-transplant DM duration 28 ± 9 years). Cardiovascular autonomic reflex tests included heart rate variation with deep breathing (I-E), Valsalva ratio (VR) and heart rate (30:15) and systolic blood pressure (Δ sBP) responses to standing. Vibration perception thresholds (VPT; biothesiometer) were used for quantitative sensory testing. Electrophysiology data analyzed were sensory (median and sural) and motor (median, tibial and peroneal) nerve conduction velocities (NCV) and peak action potential amplitudes. ENF counts, AFT and QST were also performed in 14 sex- and age-matched healthy controls (C). The Mann-Whitney U test and Wilcoxon test were used for statistical analysis.

Results: At follow-up, SPK recipients were insulin-independent with excellent glycemic control (HbA_{1c} 37 ± 3 mmol/mol) and kidney graft function (S-creatinine 102 ± 2 μ mol/L; eGFR 61 ± 17 ml/min). However, the severe ENF

depletion present at baseline (SPK vs. C: 0.8 ± 1.3 vs. 11.4 ± 4.2 ENF/mm skin surface; $p < 0.001$) was not improved at follow-up (1.4 ± 4.7 ENF/mm; $p > 0.05$ vs. baseline) with total ENF absence in 11 biopsies. Similarly, all AFT and QST results were clearly abnormal in SPK recipients in comparison with C and no amelioration occurred after long-term normoglycemia. Although some improvement was seen at follow-up in several electrophysiological parameters, statistical significance for the SPK group as a whole was achieved in median motor NCV only (median; interquartile range, pre- vs. post-SPK: 47.5; 43.0 to 50.0 vs. 51.4; 50.9 to 55.3 m/s; $p = 0.004$).

Conclusion: Lower limb epidermal nerve fibre depletion and neurological function tests - except for median motor nerve conduction velocity - were not significantly improved following establishment of long-term normoglycemia in pancreas/kidney transplant recipients. These results confirm the poor reversibility of advanced structural and functional changes in diabetic peripheral neuropathy.

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Effect of simultaneous kidney-pancreas transplantation in patients with type 1 diabetes to stabilise / progression of complications

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Background and aims: To evaluate the effect of successfully simultaneous kidney- pancreas transplantation (SPK) in patients with type 1 diabetes mellitus (T1DM) to stabilize/progression of complications.

Materials and methods: The study included 8 patients on standard immunosuppressive triple therapy. The average duration of T1DM 25 years [20;5;25], the duration of diabetic nephropathy (DN) - 7,96 лет[6;8;9,0]. All patients (3 men and 5 women) remained in the study for at least months [12;25,5] after the transplantation.

Results: The mean level of HbA1c in the group before the study was 8,65 % [8,4;9,1], then decreased to individualising glycemic targets - 5,75% [5,55;6,0] after SPK. According to a continuous glucose monitoring system using «iPRO2» marked euglycemia during the day (glycemia 3,9-8,9 mmol/l - 89 %, to lower than 3,9 mmol / l - 11% , higher than 8,9 mmol/l - 0 % of the time of day). The examination determined normoalbuminuria, GFR 80,125 [71;90,5]. All patients had normal levels of hemoglobin 120,125 [112,5;130,0], parathormone 64,94[61,37;67,5], phosphorus 1,2 [1,05;1,4], blood pressure 110[110,0;111,5]. The progression of initial proliferative diabetic retinopathy (DR) in the post-transplantation period was observed in 37.5 % of patients, followed by performing a vitrectomy and additional sessions laser panretinal photocoagulation. At 87.5% (7 people) identified nonstenotic atherosclerosis of the lower extremities, 1 patient - significant stenosis of the popliteal, posterior tibial artery to the right, requiring holding endovascular balloon angioplasty and stenting. In 75 % (6 people) developed ulcerative defects in the lower limbs and 4 people observed the progression of the chronic stage of osteoarthropathy.

Conclusion: In addition to the recovery of renal function and euglycemia in patients with T1DM undergoing SPK, noted the progression of DR and diabetic foot syndrome, which bear witness to the genesis of multivariate diabetic complications requiring verification and timely therapy.

PS 025 Hypoglycaemia rates and their economic burden

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Self-reported hypoglycaemia: a global study of 24 countries with 27,585 insulin-treated patients with diabetes: the HAT study

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Background and aims: Hypoglycaemia is an important concern for patients with diabetes and physicians when setting glycaemic targets. The Hypoglycaemia Assessment Tool (HAT) study, the largest and most comprehensive of its kind, assessed self-reported hypoglycaemia and associated predictive factors in a global population of patients with insulin-treated diabetes.

Materials and methods: HAT was a non-interventional, multicentre, 6-month retrospective and 1-month prospective study of hypoglycaemic events in 24 countries using self-assessment questionnaires and patient diaries (for 28 days) in people aged ≥ 18 years with type 1 (T1D) or type 2 (T2D) diabetes using insulin for ≥ 12 months attending routine clinics. Associations between predictive factors and hypoglycaemia were examined using negative binomial regression models adjusted for period and country.

Results: 27,585 patients completed the study (Table 1). 83.4% of patients with T1D and 50.8% of patients with T2D experienced ≥ 1 hypoglycaemic event in the 4 weeks before baseline (51.5 and 16.5 events per patient year). Higher ($p < 0.001$) incidence rates were reported in the 4 weeks after baseline (73.3 [T1D] and 19.3 [T2D] events per patient year). A greater percentage of patients with T1D vs T2D reported any (83.0 vs 46.5%), nocturnal (40.6 vs 15.9%) or severe (14.4 vs 8.9) hypoglycaemia in the prospective period. A weak correlation was seen (irrespective of diabetes type) between incidence of any hypoglycaemia and duration of diabetes (incidence rate ratio 1.01, 95% CI 1.01; 1.02) or duration of insulin therapy (1.04, 95% CI 1.03; 1.04).

Conclusion: In this large, multinational population of patients with T1D or insulin-treated T2D, rates of overall, nocturnal and severe hypoglycaemia were higher than previously reported. An increased incidence of overall hypoglycaemia in the prospective study indicated significant under-reporting of hypoglycaemia.

Characteristic	T1D (n=8022)	T2D (n=19563)				
Baseline characteristics						
Sex male/female, %	46/52	52.9/47.5				
Mean age, years (SD)	42.1 (15.1)	60.8 (10.9)				
Duration of diabetes, years (SD)	17.6 (12.0)	13.7 (8.2)				
Symptoms + BG monitoring used to define hypoglycaemia (%)	49.1	42.3				
	Retrospective	Prospective				
	T1D	T2D				
Retros. vs. prosp. (RR [95% CI])						
	T1D	T2D				
Hypoglycaemia incidence, events per patient year						
Any [4 weeks]	51.5	16.5	73.5	19.3	1.47 [1.41, 1.53]	1.20 [1.15, 1.24]
Nocturnal [4 weeks]	16.2	5.8	11.3	3.7	0.72 [0.68, 0.78]	0.69 [0.65, 0.74]
Severe	2.8*	0.9*	4.0**	2.5**	1.13 [0.99, 1.29]	1.19 [1.07, 1.32]
Hypoglycaemia prevalence, % of patients						
Any [4 weeks]	83.4	50.8	83.0	46.5		
Nocturnal [4 weeks]	46.8	21.5	40.6	15.9		
Severe	26.5*	15.8*	14.4**	8.9**		

*six month period; **4 week period; IRR, incidence rate ratio; T1D, type 1 diabetes; T2D, type 2 diabetes; IRR calculated from comparisons analysis not incidence and prevalence calculated from full analysis set

Table 1—Baseline characteristics and hypoglycaemia rates in retrospective and prospective periods

Clinical Trial Registration Number: NCT01696266

Supported by: Novo Nordisk A/S

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Hypoglycaemia study questionnaire in patients with diabetes

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Background and aims: To identify the frequency of self-reporting Moderate and severe hypoglycemia hypo and its relation to demographic, clinical variables, fear of hypoglycemia FoH and mood among adults with diabetes in outpatients clinics in United Arab Emirates.

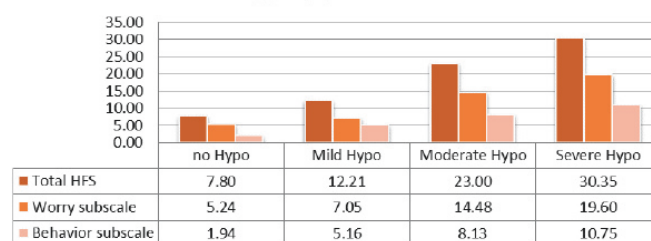
Materials and methods: The study adapted hypoglycemia patient questionnaire by ADA/Endocrine society 2013 workshop on hypo. After obtaining approval of hospital ethical committee, patient verbal consent, adults' patients with diabetes attending the clinics were encouraged to participate. FoH was assessed by Hypoglycemia fear survey HFS-II. WHO-5 survey is conducted for mood assessment when total HFS-II score ≥ 9 . Hypoglycemia incidence was grouped according to its impact on patient's function into two groups; mild / no hypo and moderate/severe hypo group. Analysis was done using SPSS 21 for windows. Comparison between quantitative variables used student T-test or ANOVA when applicable and Chi-square test for qualitative variables.

Results: Total study sample is 104 patients with diabetes, mainly DM2 92.3% 96/104.59.6 % (42) females and 40.2% (62) males. Mean age, BMI, diabetes duration and HbA1c; 45.88 \pm 9.6, 30.88 \pm 6.04, 7.68 \pm 5.89, and 7.60 \pm 1.33 respectively. 56.7% reported mild/no hypo 59/104 versus 43.3 % (45) mod/ severe hypo. Diabetes duration increased the incidence of moderate/severe hypo; 6.41 \pm 5.71 (59) vs 9.36 \pm 5.75 (45) for no/mild hypo and mod/severe hypo respectively p= 0.002 but not for the other variables; age, HbA1c, BMI, RBG. Total HFS-II and its sub-scale worry and behavior scores were significantly higher among those subjects reporting mod/severe hypo; 26.47 \pm 22.7, 16.86 \pm 15.1, 9.35 \pm 9.44 respectively vs 9.83 \pm 11.57, 6.19 \pm 7.66, 3.64 \pm 6.77 for with mild/no hypo group P<0.001. Median score for total HFS-II in the sample is 10. An analysis using the cutoff more than 10 versus \leq 10 was statistically significant in differentiating mod/severe hypo group from no/mild hypo 34.9% vs 72.2% p= 0.001. In the subgroups of female gender versus male, the mean and median for the HFS worry subscale score was higher in the female

subgroup compared to the male group; 16.25 \pm 14.40, 13 and 8.61 \pm 11.54, 4.5 p=0.005 respectively. The latter reflects a higher fear of hypo among females in the sample. A higher score but not significant in WHO-5 survey reflects a better mood among mild/no hypo 99.94 \pm 5.11 compared to mod/severe hypo group 59.87 \pm 16.8 (p= 0.092). Driving and diabetes; 31.7% and 6.1% of questionnaire respondents are checking their B.G sometimes and always before driving while they are on anti-hyperglycemic medications. 50% of insulin users are not doing so at all and 44.4% and 5.65% reported performing blood glucose checking sometimes and always before their journey. 15.1% 13/104 reported hypo while driving in the last one year.

Conclusion: The ADA/Endocrine society hypoglycemia questionnaire is a useful tool for self-reporting hypoglycemia. FoH is significant among patients with moderate/severe hypo and can be effectively highlighted by HFS-II. More educational efforts are needed to ensure safe driving in patients with diabetes in UAE.

Score of Tear FHS subgroup of Hypoglycemia



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Effect of gender on hypoglycaemia rates and perception: analysis from the SAVOR-TIMI 53 trial

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Background and aims: Some studies suggest differences in gender should be considered when individualizing treatment for people with type 2 diabetes mellitus.

Materials and methods: We studied the effects of gender on the risk for hypoglycemia and its perception in the SAVOR-TIMI 53 trial, a randomized controlled trial in which 16,492 patients with type 2 diabetes mellitus were randomized to saxagliptin or placebo and were followed for a median of 2.1 years. Patients were requested to record hypoglycemic episodes which were defined based on symptoms suggestive of hypoglycemia that recovered by carbohydrate ingestion and/or documentation of low blood glucose < 54 mg/dl (minor) or the need for assistance from another person (major).

Results: A total of 11,037 (66.9%) males and 5,455 (33.1%) females were included in the trial. Hypoglycemic events were reported by 16.2% of men and 16.1% of women; however, the event rate was higher in men (4.1 vs. 3.6 events/subject throughout follow up, p=0.002). Women were more likely than men to report clinical symptoms associated with hypoglycemia (83% vs. 76% of events, p<0.01), to require intervention (64% vs. 55%, p<0.001) which was generally oral glucose, or to need assistance from another person (3.3% vs. 2.6%, p=0.05). Missing a meal was the most frequently identified trigger for hypoglycemia and was more common in women than in men (24% vs. 16%, p<0.001). Finger stick glucose levels were reported in 83.0% and 81.5% of episodes by men and women respectively (p=0.6). Women reported hypoglycemic events at higher glucose levels than men. Glucose levels \geq 63mg%

were documented in 15% of hypoglycemic events reported by women and in 11% of episodes reported by men ($p<0.001$). Low ($<63\text{mg/dl}$) blood glucose levels were reported in 72% of the hypoglycemic episodes in men and 67% in women ($p=0.02$).

Conclusion: These findings indicate significant gender differences in the perception of hypoglycemia. The mechanisms and impact of these findings on diabetes management need further study.

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Risk and protective factors for severe hypoglycaemia in patients with type 1 diabetes

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Background and aims: Severe hypoglycaemia (SH) is associated with a critical prognosis. In addition to its acute life-threatening potential, SH may increase cardiovascular and all-cause mortality irrespective of diabetes type. Therefore, preventing from SH is a crucial challenge which requires better understanding of the predisposing risk factors. Our aim was to characterize the clinical circumstances of SH in patients with type 1 diabetes (T1DM) in a German population under the conditions of real life and to evaluate corresponding risk factors.

Materials and methods: Prospective population-based, observational trial and case control study. All SH occurring between 2007 and 2013 in a large tertiary care hospital in the Lippe-Detmold area (200.000 inhabitants) were captured. The clinical characteristics of patients with T1DM and SH were compared with an unselected regional control group of 165 patients with T1DM who did not experience SH in the seven-year period. SH was defined as a symptomatic event requiring treatment with intravenous glucose or administration of glucagon and being confirmed by a blood glucose measurement of $<50\text{ mg/dl}$. Predictive factors for SH were analysed by a multivariate regression model.

Results: A total of 959 episodes of SH in 679 patients were registered: 37.1% of cases (356/959) were related to T1DM, 51.9% (498/959) to T2DM, 3% (29/959) to pancreoprivic diabetes and 7.9% (76/959) occurred in non-diabetic individuals. The 356 cases of SH in T1DM (initial blood glucose $31.6\pm 10.7\text{ mg/dl}$) comprised 189 subjects. 50.6% (180/356) of these hypoglycaemic episodes were related to only 29 patients who experienced ≥ 3 SH. Hypoglycaemic individuals with T1DM and the 165 subjects without SH had a similar mean age (48.0 ± 19.0 vs. 47.5 ± 17.4 years; n.s.), diabetes duration (24.7 ± 15.5 vs. 23.8 ± 15.2 years; n.s.) and comparable HbA1c values ($7.5\pm 1.3\%$ vs. $7.7\pm 1.4\%$; n.s.). Under regression analyses dementia (Odds Ratio and 95% confidence interval; OR 12.93 [1.60;104.86]), living in care homes or care by home nursing services (OR 4.84 [1.33;17.63]), use of NPH as basal insulin supply (OR 3.05 [1.31;7.07]), treatment with β -blockers (OR 2.19 [1.20;4.00]), impaired awareness of hypoglycaemia (OR 2.16 [1.14;4.10]), hyperlipoproteinemia (OR 1.95 [1.09;3.48]) and extensive comorbidities (OR 1.48 [1.25;1.75]) were significant risk factors for SH (all $p<0.05$). In contrast, use of short acting insulin analogues (OR 0.29 [0.17;0.49]), higher frequency of blood glucose self measurement (OR 0.43 [0.23;0.79]) and female sex (OR 0.61 [0.39;0.96]) appeared to be protective against SH (all $p<0.05$).

Conclusion: Individuals with T1DM and SH clearly distinguish from those without SH as they are characterized by a cluster of risk factors and personal circumstances. At least, some of these predictive factors can be influenced by individualized therapies. In particular, the use of insulin analogues could be beneficial to prevent SH. The high proportion of patients with recurrent SH remains a therapeutic challenge.

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The influence of new European Union driver's licence legislation on reporting of severe hypoglycaemia by patients with type 1 diabetes

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Background and aims: The risk of losing privileges such as driver's licence may hamper reporting of severe hypoglycaemia by patients with type 1 diabetes and result in concealed severe hypoglycaemia. We tested the hypotheses that the implementation in Denmark of new stricter European Union (EU) legislation on driver's licensing with the purpose to improve traffic safety in January 2012 has reduced the self-reported rate of severe hypoglycaemia in a routine clinical setting and that anonymous reporting results in higher event rates.

Materials and methods: A cohort of 309 patients with type 1 diabetes were recruited from our outpatient clinic. Numbers of severe hypoglycaemic events defined by need for treatment assistance from another person were retrieved from medical records in the years 2010 to 2012 and retrospectively reported in an anonymous questionnaire. Data from medical records in 2012 were compared with those from 2010 and 2011 and with data from questionnaire.

Results: Reported rates of severe hypoglycaemia in the medical records were reduced by 55% in 2012 compared to the prior years ($p=0.034$). The fraction of subjects reporting recurrent episodes was grossly reduced from 5.6% to 1.5% ($p=0.014$). Compared to reporting in the questionnaire the rate of severe hypoglycaemia in 2012 was 70% lower ($p<0.001$).

Conclusion: Reporting of severe hypoglycaemia by patients with type 1 diabetes is significantly reduced following implementation of EU driver's licensing legislation, primarily due to a marked reduction in the fraction of subjects reporting recurrent episodes that according to the new regulations implies withdrawal of driver's licensing. The growing burden of concealed severe hypoglycaemia may impair the safety of affected patients and unintentionally paradoxically reduce the general traffic safety.

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Risk factors and economic burden of hypoglycaemia in patients with type 2 diabetes mellitus initiating basal insulin in a U.S. managed care system

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Background and aims: A retrospective study assessing risk factors of hypoglycemia and associated economic burden, in terms of healthcare utilisation and expenditures perspective, in adult patients with Type 2 Diabetes Mellitus initiating basal insulin on oral antidiabetic drug during 2006 to 2012 from a U.S. national claims database (Innovus LabRx).

Materials and methods: All patients had 24 months of continuous healthcare coverage with 12 months before (Baseline) and 12 months after (Follow-up) the initiation of basal insulin. Logistic regression was adapted to identify risk factors of hypoglycemia occurred at 1-year Follow-up, adjusting Baseline patient characteristics including age, gender, comorbidities, prescriptions, hypoglycemia status and healthcare utilisation. The economic burden of hypoglycemia was evaluated accounting for Baseline patient characteristics with a generalised liner model assuming gamma distribution with long link for healthcare costs. The subpopulation of patients without sulfonylurea or beta-blocker was also evaluated.

Results: While 6.8% (719/10,607) of the basal insulin initiator cohort (mean age 54 years, 44% females) had at least one medical claim for hypoglycemia, 5.9% had hypoglycemia in patients who did not use sulfonylurea or beta-blocker, and 9.8% in patients who were 65 years or older. Hypoglycemia was strongly associated with baseline hypoglycemia (Odds Ratio (OR)=5.78, 95% CI: 4.68-7.14) as well as baseline comorbidity (Charlson Comorbidity Index: 3.4 for patients with hypoglycemia vs. 2.4 for patients without hypoglycemia), microvascular disorder (OR=1.36, 95% CI: 1.12-1.66), macrovascular disorder (OR=1.33, 95% CI: 1.09-1.62), pancreatitis (OR=1.62, 95% CI: 1.06-2.47), foot exam (OR=2.93, 95% CI: 2.28-3.76), and hospitalisation (OR=1.30, 95% CI: 1.07-1.58). During Follow-up, patients with hypoglycemia vs. those who

did not, incurred a total healthcare cost of \$36,680 (95% CI: \$34,245–\$39,288) vs. \$16,850 (95% CI: \$16,549–\$17,156), diabetes medical costs (\$10,350 vs. \$2,403; $p<0.01$), and total number of inpatient days (19.8 vs. 3.4; $p<0.01$). An average hypoglycemia related hospitalisation cost was \$20,838. For every hypoglycemia episode avoided, an average Medical cost of \$733 can be saved. A 10% risk reduction in hypoglycemia was associated with an annual Medical saving of \$496.

Conclusion: Medically attended hypoglycemia was associated with and could be predicted by pre-existing comorbidity and result in increased healthcare utilisation and expenditures. An alternative basal insulin treatment with reduced risk of hypoglycemia can lessen economic burden.

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Severe hypoglycaemia often results in hospital visits and ambulance calls regardless of insulin regimen

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Background and aims: Severe hypoglycaemia is a serious complication associated with glucose-lowering treatment, in particular insulin and sulphonylureas, resulting in significant morbidity, and incurring considerable financial cost. Resource utilisation associated with severe hypoglycaemia was assessed across three insulin regimens: patients with type 2 diabetes (T2D) treated with basal insulin plus oral therapy (T2BOT), patients with T2D treated with multiple daily injections (T2MDI) and patients with type 1 diabetes (T1D) treated with basal-bolus insulin (T1BB) in a large clinical development programme.

Materials and methods: Data relating to severe hypoglycaemic events (defined as episode in which the patient required external assistance for recovery) from the insulin degludec (IDeg) and insulin degludec/insulin aspart (IDegAsp) phase 3 programme (14 trials), which included the comparators insulin glargine (IGlar), biphasic insulin aspart (BIAsp), insulin detemir (IDet) and sitagliptin, were analysed using descriptive statistics. Mealtime insulin aspart (IAsp) was used in some regimens. This analysis used the clinical trial serious adverse events records, which documented whether an ambulance/emergency response team, a hospital/emergency room visit for ≤ 24 hours, or a hospital visit of >24 hours were required.

Results: In total, 536 severe hypoglycaemic events were analysed, of which 157 (29.3%) required an ambulance/emergency team, 64 (11.9%) led to hospital/emergency room attendance of ≤ 24 h and 36 (6.7%) required hospital admission (>24 h) (Table). Although the number of events were lower in patients with T2D compared with T1D, the proportion resulting in hospital treatment for ≤ 24 h were similar across treatment regimens, whereas a higher proportion (47.6%) in the T2BOT group required hospital treatment for >24 h vs T1BB (5.0%) and T2MDI (5.3%). This resource utilisation and associated financial burden are significant: the UK tariffs for "Admitted Patient Care & Outpatient Procedures - Diabetes with Hypoglycaemic Disorders" are £1,269 for ≤ 69 years and £2,187 for ≥ 70 years, in addition to £235 for an ambulance transfer. This yields an average cost per event across treatment regimens of £305 ((11.9%+6.7%)*£1269+29.3%*£235) for patients ≤ 69 and £476 ((11.9%+6.7%)*£2187+29.3%*£235) for patients ≥ 70 years

Conclusion: This analysis suggests that severe hypoglycaemic events often result in emergency/ ambulance calls and treatment in hospital, and was observed with all insulin regimens, incurring a substantial economic burden. Thus, preventative measures to reduce severe hypoglycaemia are likely to reduce this burden.

	Treatment regimen	Number of severe hypoglycaemic events	Treatment without resource use	Ambulance/emergency calls	Hospital/emergency room treatment ≤ 24 h	Hospital admission >24 h
T1BB	IDeg + IAsp IGlar + IAsp IDet + IAsp IDegAsp + IAsp	420	62.1% (261/420)	31.0% (130/420)	9.5% (40/420)	5.0% (21/420)
T2BOT	IDeg IGlar	21	42.9% (9/21)	14.3% (3/21)	9.5% (2/21)	47.6% (10/21)
T2MDI	IDeg + IAsp IGlar + IAsp BIAsp twice daily IDegAsp once/twice daily	95	54.7% (52/95)	25.3% (24/95)	23.2% (22/95)	5.3% (5/95)
All		536	60.1% (322/536)	29.3% (157/536)	11.9% (64/536)	6.7% (36/536)

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Derivation and validation of a risk prediction tool for hypoglycaemia in hospitalised medical patients

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Background and aims: There is no objective way of identifying, on admission to hospital, those patients with diabetes who are at risk for hypoglycaemia. At present, most admitted patients with diabetes have their blood glucose monitored frequently but many never have glucose readings below an acceptable range. A method of identifying those at greatest risk of hypoglycaemia would lead to efficiencies in care delivery and improvements in quality of care and patient safety. The aim of this study was to develop and validate a tool using data available at the time of admission to predict the risk of hypoglycaemia during hospitalisation.

Materials and methods: A derivation cohort was identified of 300 randomly selected patients with diabetes admitted to a medical inpatient unit at a tertiary care teaching hospital in Toronto, Canada between November 2009 and October 2010. All point-of-care glucose tests were collected, and hypoglycaemia was defined as any result during the hospitalisation ≤ 3.9 mmol/L. Pre-specified candidate variables were abstracted from hospital records for each patient. Multivariable logistic regression was used to identify the independent predictors of hypoglycaemia using a backwards stepwise elimination method. The regression coefficients from the model were converted into an integer points score, and receiver operative characteristic curves were used to determine the threshold score that maximized sensitivity and specificity. The model was tested in a validation cohort of 300 randomly selected patients with the same inclusion criteria from a different Toronto tertiary care teaching hospital. Discrimination of the model was assessed using the c-statistic, and calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test.

Results: In the derivation cohort, 105 (35%) patients had hypoglycaemia during their hospitalisation. The predictor variables remaining after backwards elimination were sulphonylurea use, insulin use, beta-blocker use, alpha-blocker use, an emergency department visit for any reason in the prior 6 months, fever and serum creatinine. The integer points scores derived from the model are shown in the Table. A summary score of ≥ 15 had a sensitivity of 79% and a specificity of 55% for predicting hypoglycaemia. In the validation cohort, this model had a c-statistic of 0.703 and the Hosmer-Lemeshow test had a p value of 0.50.

Conclusion: We have derived and validated an easy-to-use index that could be applied at the time of admission, which is moderately discriminative for predicting hypoglycaemia in hospitalised medical patients with diabetes.

Variable	Points
On a sulfonylurea	12
On insulin	5
On a beta-blocker	4
On an alpha-blocker	4
Emergency department visit (in prior 6 months)	17
Temperature on admission $\geq 38^{\circ}\text{C}$	21
Serum creatinine ($\mu\text{mol/L}$)	
≤ 99	0
100 to 139	1
140 to 199	2
≥ 200	3

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The 'Local Impact of Hypoglycaemia Tool (LIHT)' for estimating the economic impact of hypoglycaemic episodes in national, local and user-defined populations

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Background and aims: Insulin is the most efficacious glucose lowering therapy for patients with diabetes, however, a common short-term adverse effect of insulin therapy is hypoglycaemia. Hypoglycaemia has a major impact on patients' lives affecting employment, driving, relationships, travel, and leisure activities. Severe and non-severe hypoglycaemic episodes also represent a substantial and often under-estimated cost burden to the National Health Service (NHS) and society through increased treatment costs and reduced productivity. The purpose of the Local Impact of Hypoglycaemia Tool (LIHT) is to help clinicians and budget holders estimate the cost of hypoglycaemia in the UK and specifically in their locality at the Primary Care Organisation (PCO), General Practice (GP) or user defined population level.

Materials and methods: The LIHT is a versatile model which allows the incorporation of real-life local data. The user selects a region/population of interest and based on the epidemiology of diabetes in the UK the model estimates the number of insulin-treated adults with type 1 and type 2 diabetes in that region. Using hypoglycaemia rates from the UK Hypoglycaemia Study Group (UKHSG) observational study, and the cost of a hypoglycaemic episode, the annual cost of severe and non-severe hypoglycaemic episodes in that population is estimated. The cost of a hypoglycaemic episode is dependent on the utilisation and unit costs of healthcare resources (blood glucose testing, glucose preparations, health care professional consultations, ambulance and hospital costs, derived from MIMS, Personal Social Services Research Unit (Health & Social Care) and NHS Tariff information), and is estimated to range from £1.67 to £2,195. In addition to the direct costs incurred by the NHS, indirect costs associated with lost productivity are estimated.

Results: The model highlights the cost burden of hypoglycaemia in the UK for insulin-treated adults with diabetes. On a national level, with a UK population of almost 67 million people, the total cost of managing hypoglycaemic episodes in insulin-treated adults with diabetes is an estimated £363.6m per year (£235.6m for severe and £128m for non-severe episodes, respectively). In addition, hypoglycaemic episodes are associated with indirect and hidden costs, such as lost productivity and higher rates of falls and fractures. On a local health economy level the model can provide estimations for each particular PCO within the UK. Using a hypothetical population of 100,000, the total cost of managing hypoglycaemic episodes is estimated to be £543,493 per year (£352,163 for severe and £191,330 for non-severe episodes, respectively). **Conclusion:** This model highlights the substantial cost burden to the NHS of hypoglycaemia in insulin-treated adults and may aid clinicians and NHS budget holders with choices regarding insulin treatments. The model offers an opportunity to explore how reducing hypoglycaemia can improve the quality of diabetes care for patients and result in potential budget savings.

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Influence of fasting duration on glucose metabolism in C57BL/6J mice during intravenous glucose tolerance test

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Background and aims: The intravenous glucose tolerance test (IvGTT) is commonly used to study insulin release and beta cell function in both humans and animal models. IvGTT is often preceded by a period of fasting and different laboratories use different protocols for this. Because the IvGTT is frequently used and a lot of conclusions are drawn from it, it is important to standardize the performance and use conditions that best reflect the situation aimed to study, e.g. fasting or starvation. In this study we wanted to investigate how different fasting time affects insulin secretion and glucose elimination during IvGTT in mice. The time durations were chosen based upon commonly used fasting times to find a fasting duration that gave the strongest insulin response and glucose elimination.

Materials and methods: 20g C57BL/6J BomTac female mice (n=5 in each group) were fasted for different times (12 hour, 4 hour and 1 hour). The mice were anaesthetized with Hypnorm/Dormicum 30 minutes prior to the experiment. A basal blood sample was taken from all the mice. Thereafter, glucose (1 g/kg) was injected in a tail vein, and six following blood samples were taken at 1, 5, 10, 20, 50 and 75 minutes. All blood samples were taken from the retro orbital plexus. Glucose (colorimetric assay) and insulin (ELISA) were analyzed in plasma.

Results: The mice fasted for 12 hours had higher basal glucose levels, compared with mice fasted for 1 and 4 hours ($p < 0.05$). This group also had a decreased insulin response (acute insulin response (AIR), 2.5 vs. 6.6 and 6.7, $p < 0.001$) after glucose administration, which resulted in a trend towards decreased glucose elimination (Kg, 1.9 vs. 2.8 and 2.7, $p = 0.06$). When comparing the groups fasted for 1 or 4 hours there was no difference in either glucose elimination or insulin response.

Conclusion: When planning in vivo experiment with mice it is important to know how they respond to different treatments such as fasting duration. In this study we show that the fasting time chosen for an IvGTT in normal C57BL/6J mice strongly effect the results, both concerning insulin release and glucose elimination we aimed to optimize the IvGTT protocol with a fasting time that gave maximal insulin release and glucose elimination. Our data suggest that 1h fasting is optimal for this. However, 12h fasting should be avoided, since it affects insulin release and glucose elimination negatively, probably as a stress response to starvation.

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Impact of glucose dosing regimens on evaluating glucose tolerance and beta cell function by intravenous glucose test in diet induced obese mice

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Background and aims: Metabolic studies require accurate measurements of islet function, in particular insulin secretion. An important aspect is the adaptation of beta cell function to insulin resistance, which is best described by the *disposition index*, derived from analyzing insulin and glucose data from an intravenous glucose tolerance test (IVGTT). One caveat when calculating this index is that basing the glucose dose on the individual's total body weight can result in large differences in the amount of glucose given to lean and obese individuals. In this study, we therefore evaluated alternative glucose dosing regimens for the determination of the impact of glucose dosing on measures of beta cell function in normal and diet induced obese (DIO) mice. **Materials and methods:** The glucose dosing regimens used for the IVGTT were either 0.35 mg per kg total body weight (BW) or per kg lean BW or a fixed glucose dose based on the average BW for all experimental mice. A total of 84 IVGTT were performed in 28 six-week old female C57BL6/JBomTac mice, 14 fed with 10% fat (CD) and 14 with a high fat diet (60% fat, HFD) for eight weeks before glucose tolerance testing. Lean body mass was determined

by dual emission x-ray absorptiometry using a PIXImus imager. IVGTTs were performed on 5 hour fasted mice. After injecting glucose in a tail vein, samples were taken from a retrobulbar intraorbital capillary plexus at 1, 5, 10, 20, 30 and 50 minutes for determination of insulin and glucose concentration. Glucose was dosed per total body mass (0.35g/kg), per lean body mass (0.35 g/lean kg) or equal for all animals (10 mg).

Results: Each regimen detected a similar decrease in insulin sensitivity in DIO mice. The different glucose dosing regimens gave, however, diverging results in regards to glucose elimination and the acute insulin response. Thus, the fixed dose regimen was the only that revealed impairment of glucose elimination in HFD (glucose elimination rate 1.4 ± 0.2 vs. 2.4 ± 0.2 %/min, $P < 0.01$), whereas dosing according to total BW was the only regimen which showed significant increases in acute insulin response in HFD mice (peak insulin concentration 1246 ± 230 vs. 480 ± 160 pmol/l, $P < 0.01$). The fixed dose glucose dosing regimen was the only one that revealed a significant decline in the disposition index value in HFD mice (0.7 ± 0.1 vs. 1.1 ± 0.1 , $P < 0.05$), which is characteristic of type 2 diabetes in humans.

Conclusion: Our results therefore show that using different glucose dosing regimens during IVGTT in DIO mice, it is possible to model different aspects of physiology which are similar to prediabetes and type 2 diabetes in humans, with the fixed dose regimen producing a phenotype that most closely resembles human type 2 diabetes.

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Beta cell-specific over-expression of CART improves beta cell function in vivo

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a regulator of islet hormone secretion, enhancing insulin secretion and lowering glucagon secretion, thereby fine-tuning blood glucose levels. CART also protects beta cells against glucotoxicity-induced apoptosis in vitro. Furthermore, beta cell CART expression is regulated by glucose and CART is up-regulated in beta cells of type 2 diabetes (T2D) patients and rodent models of T2D. The aim of this study was to examine the impact of up-regulated beta cell CART on in vivo beta cell function and survival, both crucial components for retaining normal glucose homeostasis.

Materials and methods: We generated transgenic mice that express CART under the control of the PDX1 promotor, resulting in overexpression of CART specifically in the beta cells (CARTtg). Mice (4 month, 6 months, and 10 months) were subjected to intravenous glucose tolerance test (IVGTT) to study insulin secretion in vivo. To study in vivo beta cell survival, mice were subjected to a multiple low dose streptozotocin (STZ)-treatment to induce diabetes, followed by weekly glucose monitoring and IVGTT 3 weeks after treatment.

Results: 4-6 months old CARTtg mice displayed normal in vivo insulin secretion compared to wild type (WT) littermates. However, at the age of 10 months CARTtg mice displayed increased insulin secretory capacity (acute insulin release, AIR) during an IVGTT compared to WT mice (WT: 3.3 vs. CARTtg: 6.3, $p = 0.02$). This was paralleled by improved glucose elimination (Kg) (WT: 1.8 vs. CARTtg: 2.5, $p = 0.03$). In STZ-treated mice blood glucose levels were elevated, compared to control mice already after 1 week (12.5mM vs. 7.9mM, $p < 0.001$). From 3 weeks after STZ-treatment CARTtg mice displayed lower glucose levels compared to WT mice (WT: 17.1mM vs. CARTtg: 21.7mM, $p < 0.001$) suggesting improved remaining insulin secretory capacity. This was confirmed by IVGTT showing that CARTtg mice secreted more insulin throughout the glucose tolerance test (Insulin AUC WT: 48 vs. CARTtg: 69.4, $p = 0.02$), which resulted in lower glucose levels (AUC glucose 1-20 min, WT: 642 vs. CARTtg: 557, $p < 0.05$).

Conclusion: Here we show that overexpression of CART in beta cells provokes improved insulin secretory capacity in vivo. Furthermore, we also show that increased beta cell CART restores insulin secretion and glucose homeostasis after diabetes induction, probably as a result of enhanced beta cell survival. In view of that CART is up-regulated in islets of T2D patients; our data suggest that CART is up-regulated in T2D as a homeostatic response trying to overcome hyperglycemia

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LKB1-dependent regulation of incretin actions in pancreatic beta cells by metformin and adiponectin

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Background and aims: Metformin is widely used to treat DM and improves blood glucose concentration through activation of LKB1-AMPK pathway. On the other hand, incretins, GLP-1 and GIP, raise intracellular Ca^{2+} and cAMP concentration in pancreatic β -cells, resulting in insulin secretion. Incretin and metformin are concurrently used for the treatment of type 2 diabetes and so we examined the effect of metformin on insulin secretion by incretin. We also examined the effect of adiponectin, which physiologically activates LKB1-AMPK pathway. Furthermore, we analyzed whether LKB1 plays a key role for the regulation of Ca^{2+} and cAMP by metformin and adiponectin.

Materials and methods: Insulin secretion, cAMP production, and intracellular Ca^{2+} concentration were analyzed using MIN6 cells by stimulating with GLP-1 or GIP. Amount of insulin secretion was measured with the Mouse Insulin ELISA KIT (TMB) (AKRIN-011T, Shibayagi, Gunma, Japan). For cAMP assay, MIN6 cells were transfected with pGloSensorTM20F cAMP Plasmid by NeonTM Transfection System and cAMP production was measured by luminescence spectroscopy using GloSensorTM cAMP assay (Promega, USA) reagent. Intracellular Ca^{2+} concentration was measured by the Fura-2 fluorescence-imaging analytic method using Meta Fulora video image analyzer. For the knock down analysis of LKB1, the gene-specific siRNA was transfected by Neon System.

Results: Under physiological glucose concentration (5.5mM) but not under high glucose concentration (16.7 mM), Ca^{2+} influx by GLP-1 or GIP stimulation was inhibited by metformin and adiponectin resulting in decreased insulin secretion. Furthermore, metformin and adiponectin regulated cAMP response by incretins. Knock down of LKB1 by siRNAs abrogated the effects of metformin on the action of incretins under physiological glucose concentration.

Conclusion: Metformin and adiponectin regulated both Ca^{2+} and cAMP responses raised by GIP and GLP-1 under physiological glucose concentration but not under high glucose condition. According to the knock down experiments, LKB1 is involved in cAMP production and Ca^{2+} response promoted by metformin. Now we are investigating whether knock down of LKB1 can abrogate the same effect of adiponectin.

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Kinome-wide RNAi screen identifies regulators of insulin like peptide production in drosophila melanogaster

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Background and aims: Insulin/IGF pathway plays a critical role in adjusting the growth, metabolism and lifespan of animals in response to nutrition. Aberrations in the pathway are causal for human metabolic diseases, most notably diabetes. Insulin/IGF like signaling (IIS) is highly conserved in multicellular animals, making simple genetic model systems, like *Drosophila*, useful in dissecting the genes involved in IIS. Aim of our project is to identify signaling pathways regulating production of insulin like peptides in *Drosophila melanogaster*. *Drosophila melanogaster* has eight insulin like peptides, four of which are secreted from fourteen median neurosecretory cells called insulin producing cells (IPCs). IPCs are considered to be *Drosophila* counterparts of pancreatic beta cells. They regulate metabolic homeostasis and growth of the organism. In this study, we have performed a focused tissue-specific in vivo RNAi screen to identify signaling pathways regulating insulin production in IPCs.

Materials and methods: dILP2-Gal4; UAS-GFP driver line was used for IPC specific knockdown of kinases. RNAi lines against kinases were obtained from VDRC, Austria and Bloomington Stock Center, USA. qRT-PCR assays were used to measure mRNA levels of insulin like peptides and immunostainings using anti-dILP2 antibody were used to assess secretion of insulin like peptides.

Results: 231 protein kinases were screened of which twelve genes showed severe undergrowth phenotype upon knockdown. These hits were confirmed using at least two independent RNAi lines. These genes were analyzed for

their roles in IPC viability, insulin-like peptide (dILP) mRNA expression and dILP2 secretion. None of the kinases had an effect on the viability of IPCs. One of the kinases, Tousled-like kinase, showed significantly reduced dILP3 expression. Further, knockdown of several identified genes like ADCK, PKC98E, Rio2, aPKC, CDK12 and Rio1 showed accumulation of dILP2 in IPCs, implying disturbed dILP2 secretion. One of the genes, aPKC, has previously been linked to insulin secretion in mammals, suggesting conserved function. The mechanisms of action of other genes which are not previously studied with respect to insulin signaling are being investigated.

Conclusion: In conclusion, we have identified twelve protein kinases, which regulate insulin production in *Drosophila melanogaster*. One of the genes, Tlk, regulates dILP3 transcription in IPCs. Further, ADCK, PKC98E, Rio2, aPKC, CDK12 and Rio1 play a role in secretion of insulin like peptide 2 from IPCs.

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Antidiabetic effects of tigerinin-1R in mice chronically maintained on high fat diet

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Background and aims: We previously reported in vitro and acute in vivo insulinotropic effects of tigerinin-1R (RVCSAIPICH.NH₂), an amphibian host defence peptide, isolated from the skin secretion of *Hoplobatrachus rugulosus*. The present study investigated plasma degradation and chronic metabolic effects of tigerinin-1R in Swiss NIH mice fed a high fat diet to induce obesity-diabetes.

Materials and methods: Proteolytic degradation of synthetic tigerinin-1R was investigated by reversed-phase HPLC and MALDI-TOF mass spectrometry using GLP-1(7-36) as positive control. Changes in glycaemic responses and metabolic parameters were measured in mice with high fat diet-induced obesity-diabetes treated with twice-daily intraperitoneal injection of tigerinin-1R (75nmol/kgbw) for 15 days. Glucose concentrations were measured by glucose oxidase method using an Analox GM9 glucose analyser and insulin concentrations were measured by radioimmunoassay. Indirect calorimetry and body composition were measured by CLAMS and DEXA whole body scanning. Insulin secretory responses of islets isolated from treated and untreated mice were examined.

Results: Unlike GLP-1(7-36), tigerinin-1R was resistant to *in vitro* degradation by plasma enzymes. Twice-daily injection of tigerinin-1R (75nmol/kg bw) for 15 days had no significant effect on food intake or body weight. Non-fasting glucose levels were significantly lowered (16%, $P < 0.05$) and insulin levels were elevated (20%, $P < 0.05$) compared to saline treated controls at the end of the treatment period. Glycaemic responses to oral and intraperitoneal glucose administration (180mmol/kg body weight) were improved by 26% ($P < 0.05$) and 22% ($P < 0.05$) respectively in mice treated with tigerinin-1R. Plasma insulin responses to glucose administration were also significantly enhanced by 40% ($P < 0.001$, oral) and 41% ($P < 0.001$, intraperitoneal). Treatment with the peptide had no effect on insulin sensitivity but compared to saline treated controls significantly improved beta cell responses of isolated islets in response to GLP-1 (10⁻⁶M, 1.9-fold, $P < 0.001$), GIP (10⁻⁶M, 1.6-fold, $P < 0.05$, alanine (10mM, 1.4-fold, $P < 0.001$), L-arginine (10mM, 1.6-fold, $P < 0.05$) and KCl (30mM, 2.0-fold). Oxygen consumption, CO₂ production, respiratory exchange ratio, energy expenditure and body composition were not significantly altered by treatment with tigerinin-1R.

Conclusion: These observations provide the first evidence for the therapeutic potential of tigerinin-1R as a novel anti-diabetic agent.

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Gamma-oryzanol, a unique component of brown rice, improves pancreatic islet function via inhibition of exaggerated dopamine receptor signalling

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Background and aims: We recently demonstrated that γ -oryzanol, a major bioactive component of brown rice, prevents obesity and type 2 diabetes. Gamma-oryzanol attenuated the preference for dietary fat through reduction of exaggerated endoplasmic reticulum (ER) stress in high fat diet (HFD)-induced obese mice. We also found that γ -oryzanol improved HFD-induced glucose intolerance, but the underlying mechanism still remains unclear. On the other hand, previous studies showed that local dopamine D2 receptor (D2R) signaling prevents insulin secretion in islets. In this context, we tested the potential involvement of D2R signaling in γ -oryzanol-mediated glucose homeostasis in pancreatic islets.

Materials and methods: Gamma-oryzanol was delivered into the stomach of male C57BL/6J mice by a gavage needle and its effects on islets function were determined. Response of a series of drugs influencing D2R signaling and γ -oryzanol, and insulin secretion in pancreatic β -cell line, MIN6 cells and isolated islets was assessed.

Results: In islets from HFD-fed obese mice, D2R signaling was exaggerated in islets from mice fed HFD (e.g. D2R mRNA level; 2.0-fold increase vs. chow-fed mice, $p < 0.05$), where augmented ER stress coexisted (e.g. Chop mRNA level; 2.1-fold increase vs. chow-fed mice, $p < 0.01$). In pancreatic β -cell line, MIN6 cells and isolated islets, γ -oryzanol activated the cAMP/cAMP-dependent protein kinase (PKA) pathway via inhibition of D2R signaling, thereby enhancement of glucose-stimulated insulin secretion (GSIS) (insulin secretion, 25mM glucose; MIN6 cells, 1.3-fold, $p < 0.05$, islets, 2.2-fold increase vs. vehicle-treated cells, $p < 0.01$). In contrast, exaggerated secretion of insulin-counteracting hormone, glucagon was remarkably suppressed by γ -oryzanol both in vivo and in vitro. ER stress is linked to the regulation of pancreatic β -cell functions and the pathophysiology of type 2 diabetes. ER stress in β -cells leads to apoptosis and suppression of insulin biosynthesis. We also found that γ -oryzanol reduces ER stress in islets from HFD-fed mice (e.g. Chop mRNA level; 37 % reduction, $p < 0.05$). Moreover, in MIN6 cells treated with tunicamycin, an ER stress inducer, γ -oryzanol improved in insulin secretion and prevented ER stress-induced apoptosis.

Conclusion: These data indicate that γ -oryzanol directly improves the survival and function of murine pancreatic islets. A series of the effects of γ -oryzanol on pancreatic islets may shed light on a novel, natural food-based medicine for type 2 diabetes in humans.

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Activation of GPR120 by fatty acid agonists augments insulin release from pancreatic islets and improves glucose tolerance in mice

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Background and aims: GPR120 is a rhodopsin-like GPCR that has a high affinity for long chain saturated fatty acids 14-18 carbons and unsaturated fatty acids 16-22 carbons. This aim of this study was to assess the potency and selectivity of various GPR120 agonists and determine the cellular localisation of GPR120 in clonal beta cells and pancreatic islets.

Materials and methods: Insulin secretion and alterations in intracellular Ca²⁺ and cAMP response to glucose and GPR120 agonists including endogenous agonists (ALA, EPA, DHA) and a synthetic analogue (GW-9508) were examined using clonal pancreatic BRIN-BD11 cells, mouse pancreatic islets and *in-vivo* studies using NIH Swiss mice. Cytotoxicity was assessed by LDH release. Cellular localisation of GPR120 was explored by double staining immunohistochemistry.

Results: The most potent and selective GPR120 agonist tested was ALA (EC₅₀ 1.2x10⁻⁸ mol/l) at 10⁻⁸-10⁻⁴ mol/l with a 1.5- to 2.1-fold increase in insulin

release at 5.6mM glucose ($p < 0.001$), with maximum stimulation of insulin secretion by 53% at 10^{-4} mol/l ($p < 0.001$) in BRIN-BD11 cells. Stimulation of insulin secretion was observed with GW-9508 (6.4×10^{-8} mol/l; 47%), EPA (7.9×10^{-8} mol/l; 36%) and DHA (1.0×10^{-7} mol/l; 50%) at 5.6mM glucose. At stimulatory glucose concentrations, ALA was the most potent agonist tested (EC_{50} of 8.5×10^{-8} mol/l) at 10^{-8} – 10^{-4} mol/l with a 1.2- to 1.6-fold increase in insulin release at 16.7mM glucose ($p < 0.05$ – $p < 0.001$). DHA enhanced insulin release at 10^{-7} – 10^{-4} mol/l by 1.3- to 1.7-fold ($p < 0.01$ – $p < 0.001$) (EC_{50} of 1.2×10^{-7} mol/l) while EPA augmented insulin secretion at 10^{-9} – 10^{-4} mol/l by 1.2- to 1.6-fold ($p < 0.05$ – $p < 0.001$) (EC_{50} of 1.7×10^{-5} mol/l). Synthetic agonist GW-9508 enhanced insulin secretion at 10^{-8} – 10^{-4} mol/l by 1.2- to 1.7-fold ($p < 0.05$ – $p < 0.001$) (EC_{50} of 7.0×10^{-7} mol/l). Results were corroborated by islet studies, with no evidence of cytotoxic effects. Dose-dependent insulin secretion by GPR120 agonists was glucose-sensitive and accompanied by elevations of intracellular Ca^{2+} ($p < 0.05$ – $p < 0.001$) and cAMP ($p < 0.05$ – $p < 0.01$). Immunocytochemistry demonstrated GPR120 expression on BRIN-BD11 cells and was confined to islet beta cells with no distribution on alpha cells. Administration of GPR120 agonists ($0.1 \mu\text{mol/kg}$ body weight) in glucose tolerance studies was tested in mice. Using AUC data, ALA ($p < 0.05$), EPA ($p < 0.05$) and GW-9508 ($p < 0.01$) decreased the glycaemic excursion. Similarly both EPA ($p < 0.05$) and GW-9508 ($p < 0.01$) enhanced insulin release by 35–40% after 15 min while both small molecules stimulated insulin secretion by 33–41 after 30 min ($p < 0.01$). All agonists tested with exception of DHA augmented glucose-induced insulin release ($p < 0.05$ – $p < 0.01$) when compared to glucose alone.

Conclusion: GPR120 is expressed on pancreatic beta cells, and agonists at this receptor are potent insulin secretagogues, with therapeutic potential for type 2 diabetes.

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Chronic glucocorticoid treatment induced insulin resistance but improved glucose tolerance due to increased insulin secretion and beta cell neogenesis

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Background and aims: Cortisol and corticosterone, the physiological glucocorticoids (GCs) in humans and rodents, respectively, have pleiotropic functions to modulate blood pressure, immune responses, and energy homeostasis. GCs and synthetic GCs are commonly used for the treatment of inflammatory diseases but their adverse effects can overcome their benefit, restricting their long-term use. More specifically, GCs exert deleterious effects on glucose metabolism, leading to a wide range of alterations from insulin resistance to complicated diabetes, but also induce weight gain associated with lipodystrophy.

Materials and methods: In this study, we investigated the effects of a long-term GC exposure in C57/BL6 male mice. Corticosterone (CORT) was given in drinking water ($100 \mu\text{g/mL}$) for 8 weeks. Glucose tolerance, insulin sensitivity, gene expression in adipocytes and pancreatic islets and pancreatic morphometry were studied.

Results: Mice treated with GC had a significant weight gain ($\Delta = 7.1 \text{ g}$ vs 3.0 g , $p < 0.001$) with an accumulation of both brown ($x3$, $p < 0.001$) and white ($x2$, $p < 0.01$) adipose tissues, characterized by a visceral adipocyte hyperplasia and subcutaneous adipocyte hypertrophy. Surprisingly, GC treatment promoted macrophage infiltration (F4/80, CD68) ($x5$, $p < 0.05$) within all adipose tissues. Of note, a M1/inflammatory macrophage phenotype (MCP1, IL6, TNF α) was restricted to visceral adipose tissue compared to subcutaneous fat depot, suggesting a substantial damaging effect on insulin sensitivity. Accordingly, CORT treatment induced an insulin-resistance, as demonstrated by insulin tolerance test and HOMA-IR ($x10$, $p < 0.001$) as well as an decreased glucose uptake into adipose tissue (-50% , $p < 0.01$). CORT mice exhibited also postprandial hyperglycaemia (following *ad libitum* nocturnal feeding) (259 mg/dL vs 140 mg/dL , $p < 0.001$) but this effect was lost in fasted condition due to a reduced hepatic gluconeogenesis measured by pyruvate tolerance test. Strikingly, following a 6-h-fasting, GC treatment resulted in an improved glucose tolerance (OGTT/IPGTT), due to increased plasma insulin levels ($x8$, $p < 0.001$). Pancreatic analysis revealed a dramatic increase of β -cell fraction (4.3% vs 0.6% , $p < 0.05$) and β -cell mass ($x7$, $p < 0.05$) associated with improvement in insulin secretion capacity from isolated islets. Increased islets size was observed ($x2$, $p < 0.05$), due to β -cell proliferation. More surprisingly, a dramatic increased islet density was found ($x4$, $p < 0.05$),

revealing β -cell neogenesis, a result confirmed by increased *Ngn3* expression. Several other β -cell genes (*Pdx1*, *Rfx6*, *Nkx 2.2*, *NeuroD*, *Ins*) were up-regulated, pointing to a new insight into GC-induced β -cell adaptation.

Conclusion: In this study we demonstrated that GC could differentially act on various fat depots to promote metabolic alteration and evidenced a new function of GCs on β -cell neogenesis.

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Effects of caloric restriction and Ins1 gene dosage on glucose homeostasis in female mice

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Background and aims: Therapeutic benefits of caloric restriction (CR) on clinical outcomes in treatment of neurodegenerative disease, cancer, cardiovascular disease, and diabetes have been proposed. Studies indicate the positive health outcomes produced by CR may involve cellular nutrient-sensing pathways including insulin/insulin-like growth factor 1 signalling. CR has been reported to have significant effects on glucose metabolism and body composition, lowering fasting blood glucose and insulin, as well as improving glucose tolerance and insulin sensitivity. However, it is not clear which, if any, of the effects of CR are due to the lowering of circulating insulin. To determine whether CR-improved glucose homeostasis is a function of circulating insulin-dependent mechanisms we performed a gene-dosage study with mice either wildtype or heterozygous at the insulin 1 gene locus, while lacking the insulin 2 gene entirely. We have previously shown that male *Ins1+/-:Ins2-/-* mice are protected from diet-induced obesity compared to *Ins1+/+:Ins2-/-* littermate controls. In the present study, using female mice of the same genotypes, we compared how changes to caloric intake and insulin gene dosage affect circulating hormone levels, glucose homeostasis, energy expenditure, markers of healthy aging, and longevity.

Materials and methods: Female *Ins1+/-:Ins2-/-* mice and *Ins1+/+:Ins2-/-* littermate controls were fed a chow diet *ad libitum* (AL) until 13 weeks of age, then half of the mice continued AL feeding, while the others were placed on a CR diet where they were fed 60% of what their genotype-matched littermate controls ate daily. All mice were singly housed and CR mice were fed at night. Body mass, insulin secretion, and blood glucose response to intraperitoneal injections of glucose or insulin were assessed over a 1-year period.

Results: With the onset of CR, body mass in both genotypes fell and reached a new equilibrium by 20 weeks of age. Until ~38 weeks of age, food intake of AL-fed and hence CR fed *Ins1+/-:Ins2-/-* mice was modestly higher than that of *Ins1+/+:Ins2-/-*, leading to a small difference in mean body weight of CR mice that disappeared as food intake of the two genotypes converged. As expected, mice on CR had lower fasting and fed plasma glucose when compared to genotype controls ($p < 0.05$). CR improved glucose tolerance in mice at 25 and 51 weeks of age, irrespective of *Ins1* gene dosage. All groups had comparable glucose-stimulated insulin levels at 27 and 53 weeks of age. However, we observed paradoxical effects of CR on insulin sensitivity. While the first 30 minutes post-injection were similar across groups, CR mice of both genotypes exhibited a rapid recovery to baseline glucose. Fasting insulin levels at 53 weeks were also paradoxical, with *Ins1* gene dosage having no effect in CR mice. In fact, CR *Ins1+/-:Ins2-/-* mice had significantly increased circulating insulin ($p < 0.05$) when compared to AL control. CR *Ins1+/+:Ins2-/-* mice exhibited the expected reduction in circulating insulin compared to AL control (0.21 ± 0.08 versus $0.31 \pm 0.07 \text{ ng/mL}$; $p = 0.0575$).

Conclusion: Our results suggest one *Ins1* allele is able to produce equal amounts of insulin as two alleles, although if a stressor is applied, such as ageing, this may be abrogated. CR appears able to prevent this loss and cause a rapid blood glucose recovery after exogenous insulin in a manner not observed in other studies.

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PS 027 Determinants of insulin secretion in humans

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Across glucose tolerance spectrum, men display greater decreases in insulin secretion than women

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Background and aims: Little is known about gender differences in insulin secretion (IS). As part of a project examining the utility of beta cell function tests, we studied effect of gender on IS response to the arginine (ARG) stimulation test (AST) and the mixed meal tolerance test (MMTT) in overnight fasted, obese men (M) and women (W) with normal glucose tolerance (NGT), prediabetes (PDM) and type 2 diabetes (T2DM).

Materials and methods: For AST, acute IS (AIRarg) was measured over 5 min at baseline glucose after an i.v. ARG bolus (5 gm over 30 sec). Immediately following these samples, a 60 min infusion (900 mg/min) of glucose was initiated; ARG was administered again after 50 min of the glucose infusion; IS samples were then repeated (AIRmax). All arg results are baseline-adjusted. For MMTT, IS (Φ tot and DItot) were measured in response to a standardized 450 kcal meal and estimated using minimal model.

Results: The table below summarizes the results. Within each gender significant declines in insulin secretion were detected for all 4 parameters (NGT and PDM were largely similar v. T2DM). To compare the changes in insulin secretion of women to men across the glucose tolerance groups, an ANCOVA model was developed. Of covariates tested (BMI and age), only age was included in the model as a significant covariate. ANCOVA showed that from NGT to T2DM, as seen in both the AST and MMTT, the decline in insulin secretion is greater in men than in women.

Conclusion: We conclude that 1) in a cross-sectional analysis of men and women with NGT, PDM, and T2DM, insulin secretion declines in both genders; 2) From NGT to T2DM, men show a greater decline than women in 2 different tests of insulin secretion. These results may have implications for gender balance in study designs measuring insulin secretion.

	Age (Yr) (mean±SD)	BMI (kg/m ²) (mean±SD)	Baseline Glucose (mg/dL) (mean±SD)	ARGININE TEST		MMTT	
				AIRarg (pM)	AIRmax (pM)	MMTT Φ tot (10 ⁻³ /min)	MMTT DItot (10 ⁻¹³ /(μ U/ml)(min ²))
NGT (W) N=11	42.6±9.4	31.0±2.4	5.2±0.3	511 (423-617)	2440 (1828-3256)	93.0 (77.4-111.6)	478.4 (380.8-601.1)
NGT (M) N=12	41.2±7.4	31.8±3.2	5.2±0.3	666 (576-770)	3060 (2549-3673)	112.4 (90.0-140.4)	450.4 (312.2-649.6)
PDM (W) N=6	45.5±12.4	32.4±1.4	6.0±0.6	603 (478-761)	2520 (2132-2978)	106.5 (90.0-126.1)	260.0 [^] (193.2-349.8)
PDM (M) N=2	49.0±2.3	31.6±0.8	6.4±0.1	806 (376-1726)	2914 (2070-4101)	99.4 (70.2-140.8)	188.1 (78.2-452.4)
T2DM (W) N=11	56.8±7.0	32.6±3.9	8.7±1.2	303 [^] # (234-393)	717 [^] † (542-948)	19.1 [^] † (14.4-25.4)	33.6 [^] † (21.5-52.3)
T2DM (M) N=11	52.5±8.6	32.9±3.9	8.7±1.6	201 [^] † (161-250)	489 [^] † (387-618)	12.4 [^] † (9.9-15.7)	9.0 [^] † (5.4-15.0)
ANOVA WITHIN women OR men for IS and across populations. Within M and W (but not between): [^] p < 0.05 vs. NGT, [†] p < 0.001 vs. NGT, [‡] p < 0.001 vs. PDM, [#] p < 0.01 vs. PDM				WOMEN: p=0.01 MEN: p<0.001	WOMEN: p<0.001 MEN: p<0.001	WOMEN: p<0.001 MEN: p<0.001	WOMEN: p<0.001 MEN: p<0.001
ANCOVA analysis comparing the change in IS BETWEEN men and women across GT				p < 0.05	p = 0.08	p < 0.05	p < 0.05

Clinical Trial Registration Number: NCT01663207

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Circulating non-esterified fatty acids (NEFA) do not associate with beta cell dysfunction: evidence from the RISC study

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Background and aims: It is commonly held that chronically elevated NEFA levels adversely affects insulin secretion and insulin action (lipotoxicity). Although the association between NEFA and insulin resistance is well established, the effect of raised NEFA on β -cell function has only been explored in small experimental protocols using acute elevations in NEFA. Our aim was to analyse the relationship between endogenous NEFA levels and insulin secretion and β -cell function both cross-sectionally and longitudinally.

Materials and methods: In 701 women and 566 men from the Relationship Between Insulin Sensitivity and Cardiovascular Disease (RISC) cohort (mean age 44 years, BMI range 17–44 kg/m², 14% with impaired fasting glycaemia (IFG) and 9% with impaired glucose tolerance (IGT)) followed up for 3 years, we measured insulin sensitivity (by a euglycaemic insulin clamp) and β -cell function (by modelling of the C-peptide response to oral glucose and as the acute insulin response (AIR) to intravenous glucose).

Results: At baseline, both fasting and insulin-suppressed NEFA were significantly ($p < 0.0001$) higher across glucose tolerance groups, while insulin sensitivity was lower ($p < 0.0001$), insulin output was higher ($p < 0.0001$) and β -cell glucose sensitivity and AIR were lower (both $p < 0.0001$). By multiple logistic analyses adjusting for center, sex, age, BMI, WHR, and glucose tolerance, both fasting and insulin-suppressed NEFA were inversely related to insulin sensitivity (both $p < 0.0001$). Furthermore, after also adjusting for insulin sensitivity, insulin-suppressed NEFA were positively associated with total insulin output ($p = 0.0042$). In contrast, in the same multivariate models neither fasting nor insulin-suppressed NEFA were related to β -cell glucose sensitivity or AIR. This result was confirmed when also accounting for insulin sensitivity or family history of diabetes. At follow up, glucose tolerance status worsened in 127 subjects. By multiple logistic regression, this change was predicted by male gender, higher age and higher waist-to-hip ratio, worse baseline glucose tolerance, lower insulin sensitivity and β -cell glucose sensitivity (area under ROC curve = 0.79). In this model, neither baseline fasting nor baseline insulin-suppressed NEFA were significant predictors of progression.

Conclusion: In the non-diabetic and prediabetic state, circulating endogenous NEFA are independently associated with impaired insulin sensitivity and enhanced insulin release but not with measures of β -cell function, and do not predict deterioration of glucose tolerance. Thus, in the RISC cohort there is no evidence for β -cell lipotoxicity of endogenous total NEFA concentrations.

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Increment of serum C-peptide by glucagon test closely correlates with human beta cell mass

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Background and aims: Evaluation of beta-cell mass is important in clinical practice of diabetes. Our objective was to evaluate the relationships between human beta-cell mass and various clinical parameters, including insulin secretory capacities.

Materials and methods: We conducted a study of pancreatic tissues obtained from 32 (19 males and 13 females) patients undergoing a pancreatectomy between 2008 and 2012 in the Department of Gastroenterological Surgery of our hospital, and who had not been treated with oral hypoglycaemic agents or insulin. They were classified into normal glucose tolerance (NGT, n=13), impaired glucose tolerance (IGT, n=9), and diabetes mellitus (DM, n=10). Their insulin secretory capacity was evaluated by homeostasis model assessment

of beta-cell function [HOMA-beta, fasting immunoreactive insulin ($\mu\text{IU/ml}$) $\times 20$ / (fasting plasma glucose (mmol/l) - 3.5)], C-peptide index [fasting C-peptide (nmol/l) / fasting plasma glucose (mmol/l)], insulinogenic index [Δ serum insulin 0-30 min (pmol/l) / Δ plasma glucose 0-30 min (mmol/l) in 75 g-OGTT] and Δ C-peptide by glucagon test [Δ C-peptide, increment of serum C-peptide (nmol/l) at 6 min after intravenous injection of 1-mg glucagon]. Pancreatic samples were fixed in formaldehyde, embedded in paraffin for subsequent analysis and cut from these paraffin blocks into 5- μm thick sections. We used immunohistochemistry to determine pancreatic beta-cell area. The relative beta-cell area represented the proportion of insulin-positive cell area to whole pancreatic section (%).

Results: The mean of relative beta-cell area was 1.072 ± 0.424 , 0.998 ± 0.419 and $0.762 \pm 0.441\%$, in NGT, IGT and DM, respectively. Δ C-peptide ($r=0.64$, $p=0.002$), HOMA-beta ($r=0.50$, $p=0.003$) and C-peptide index ($r=0.36$, $p=0.042$) correlated significantly and positively with the relative beta-cell area. Insulinogenic index tended to correlate positively but not significantly with the relative beta-cell area ($r=0.33$, $p=0.078$). Among them, Δ C-peptide showed the closest association with the relative beta-cell area. Age tended to correlate negatively but not significantly with the relative beta-cell area ($r=-0.33$, $p=0.066$). Neither BMI nor HbA1c showed a significant correlation with the relative beta-cell area ($r=0.26$, $p=0.147$; $r=-0.16$, $p=0.378$, respectively). Glucose levels at 0, 30, 60, 120 min in 75 g-oral glucose tolerance test (OGTT) tended to correlate negatively with the relative beta-cell area ($r=-0.26$, $p=0.145$, $r=-0.30$, $p=0.105$, $r=-0.31$, $p=0.087$, $r=-0.34$, $p=0.064$, respectively), while the area under the curve of plasma glucose level from 0 to 120 min in 75 g-OGTT correlated significantly and negatively with the relative beta-cell area ($r=-0.36$, $p=0.045$).

Conclusion: Δ C-peptide by glucagon test, HOMA-beta, and CPI correlated closely with beta-cell area, and Δ C-peptide was the most valuable index for the prediction of beta-cell mass.

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Normal meal tolerance test is more valuable than glucagon stimulation test in type 2 diabetes

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Background and aims: The glucagon stimulation test (GST) is a standard method of evaluating endogenous insulin secretion. However, the GST has adverse effects and takes effort. In contrast, the meal tolerance test (MTT) has no adverse effect and cheaper than GST. Moreover, to perform the MTT by normal meal is important to evaluate insulin secretion in real life. However, the MTT is affected by the glucotoxicity than GST in the hyperglycemic state. The aim of this study is to evaluate the properties of the glucagon stimulation test and normal meal tolerance test in type 2 diabetes patients.

Materials and methods: We enrolled 142 patients of type 2 diabetes (mean: age 61.0, M/F: 80/62, BMI 24.7, HbA1c 9.4) and performed the GST and the MTT. We performed the MTT by using a normal diabetes food of 30kcal/ day per ideal body weight, which contains 60% of carbohydrates, protein 20%, lipid 20%. In the MTT, patients continued oral hypoglycemic agents and insulin treatment as usual. In the MTT, we measured plasma glucose (PG) and serum C-peptide immunoreactivity (CPR), and plasma insulin level before and 120minutes after meal load. We calculated increment of CPR (Δ CPR) by subtracting fasting CPR (FCPR) from 6 minutes after glucagon injection and 120 minutes after meal load.

Results: The mean fasting PG (FPG) was 154 mg/dl, the mean FCPR was 2.2 ng/ml, the mean GST Δ CPR was 2.0 ng/ml, and the mean MTT Δ CPR was 3.1 ng/ml. 104 patients represent higher Δ CPR in the MTT than the GST, and the mean MTT Δ CPR was significantly higher than the mean GST Δ CPR ($P<0.01$). To exclude influence of antidiabetic drugs, we examined 42 subjects that did not use antidiabetic agents. The mean GST Δ CPR was 2.4 ng/ml, and the mean NMTT Δ CPR was 4.3 ng/ml and the mean MTT Δ CPR was significantly higher than the mean GST Δ CPR ($P<0.01$). To consider influence of glucotoxicity to both test, we performed ROC analysis by FPG. The optimal cut point of the FPG was under 147 mg/dl to define the MTT superior to the GST (sensitivity 0.64, specificity 0.76, AUC 0.73).

Conclusion: The MTT could examine insulin secretion including incretin effect by the meal load, and it may be one of the reasons why the MTT Δ CPR is higher than the GST Δ CPR. Therefore, the MTT could evaluate the endogenous insulin secretion ability more than the GST. However, the MTT is affected by the glucotoxicity than the GST. We had better consider to perform

the GST in the hyperglycemic state, especially FPG is 147mg/dl or more. We need to use the GST and the MTT properly by a level of the FPG. In conclusion, the MTT is more valuable as an insulin secretion test in type 2 diabetes patients than the GST.

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Adaptation of beta cell response to carbohydrate loading: influence of insulin resistance

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Background and aims: To maintain euglycemia, the β -cell must cope with variable carbohydrate intake (ChI) and with insulin resistance. Aim of this study was to quantify how insulin secretion adapts to variable ChI in insulin sensitive (IS) and insulin resistant (IR) individuals.

Materials and methods: 143 nondiabetic subjects, aged 18-55 years were classified as IS ($n=64$) or IR ($n=79$) using the euglycemic clamp. On sequential days, subjects were given a meal every 3½ hours over a 14-hour period (4 meals/day), with standard (300 kcal) or double (600 kcal) ChI in random order. Serial measurements of glucose, insulin and C peptide were obtained. Model-based total insulin secretion, β -cell glucose sensitivity (slope of the glucose concentration-insulin secretion relationship), and potentiation (fold enhancement of glucose sensitivity from baseline) were calculated.

Results: Total insulin secretion increased from 300-kcal to 600-kcal ChI in both groups, and was higher in IR compared to IS (Table). β -cell glucose sensitivity followed a similar pattern. Potentiation was also increased with the ChI load, but in IR the increase was blunted. In IR, mean glucose levels and their increase from 300-kcal to 600-kcal ChI were higher than in IS. In multivariate regression, mean glucose was significantly positively related to ChI and negatively to insulin sensitivity, β -cell glucose sensitivity and potentiation ($r^2 = 0.37$, $p<0.0001$). In IR, the percentage of plasma glucose values ≥ 11.1 mmol/L rose from 2.3% to 6% on doubling ChI, whereas it declined from 0.6% to 0.2% in the IS group.

Conclusion: Higher carbohydrate meal content elicited an insulin secretion response that was higher both in absolute terms and in relation to the glucose levels; this response was enhanced in IR. However, the enhancement of potentiation from 300-kcal to 600-kcal ChI was impaired in IR subjects, in whom the up-regulation of β -cell glucose sensitivity compensation was insufficient to preserve glucose tolerance to the same extent as in IS subjects. Therefore, even in nondiabetic subjects insulin resistance is associated with a relative incompetence of β -cell function despite insulin hypersecretion.

Parameter	IS		IR	
	300 kcal	600 kcal	300 kcal	600 kcal
Total insulin secretion (nmol·m ⁻²)	206 [75]	283 [90]	312 [113]	448 [165]
Glucose sensitivity (pmol·min ⁻¹ ·m ⁻² ·mM ⁻¹)	90 [44]	113 [55]	108 [50]	122 [67]
Potentiation (fold)	1.47 [0.71]	2.23 [1.05]	1.42 [0.54]	1.79 [1.08]
glucose AUC (mol·h)	0.66 \pm 0.07	0.69 \pm 0.12	1.02 \pm 0.06	1.59 \pm 0.10
Data are mean \pm SE or median [interquartile range]. IS vs IR and 300 vs 600 kcal intake differences are significant by two-way ANOVA.				

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Decrease in plasma mannose level after glucose load is associated with glucose tolerance: plasma mannose level as a putative indicator of glycogenolysisS. Hirano¹, K. Yoshimura¹, H. Takata¹, S. Ohmi¹, T. Taguchi², S. Yamada², I. Miwa², Y. Terada¹, S. Fujimoto¹;¹Department of Endocrinology, Metabolism and Nephrology, Kochi University, Nankoku,²Department of Pathobiochemistry, Meijo University, Nagoya, Japan.

Background and aims: Mannose is a monosaccharide constituent of glycoproteins and glycolipids. Experiments in rats showed previously that the plasma mannose level decreases after glucose load which causes insulin secretion in normal rats, but does not decrease in diabetic rats and that hepatic glycogenolysis is a source of this plasma mannose, but these results are not fully elucidated in human. Subjects with glucose intolerance have impaired secretion and sensitivity of insulin, which are the most important factors in suppression of glycogenolysis. To explore the possibility of the plasma mannose level as an indicator of glycogenolysis in human, the decrease in plasma mannose levels after glucose load in subjects with various degrees of glucose intolerance was examined and was analyzed to clarify association with clinical factors.

Materials and methods: 75g OGTT was performed on Japanese subjects without diabetic medication from whom informed consent was obtained. Based on OGTT data, subjects were classified as normal (NGT), impaired glucose tolerance (IGT), and diabetes (DM) according to WHO criteria. In each group, 25 subjects were consecutively recruited [total 75 subjects, age: 65±11 (mean±SD); male/female: 34/41; BMI: 24.9±3.8 (mean±SD)]. Insulinogenic index (IGI) as an index of insulin secretion and QUICKI and Matsuda index as indices of insulin sensitivity were calculated. Mannose was assayed using established method. Briefly, after labeling with 4-aminobenzoylethyl ester, the concentration of mannose was determined using HPLC. Glucose contained in samples was confirmed not to affect the assay. The study protocol was approved by the ethics committee of the institute.

Results: Plasma mean levels of glucose during 120 min (Gm) were significantly different among groups [NGT: 138±20, n=25; IGT: 178±27, n=25; DM: 226±24 mg/dl, n=25, (means±SD)], while mean IRI during 120 min (Im) did not significantly differ. Basal levels of mannose were similar among groups (NGT: 40.8±10.0; IGT: 43.7±11.2; DM: 45.0±12.0 µM). After glucose load, the plasma mannose level was decreased gradually and reached plateau at 90 min in NGT, but was not decreased significantly in DM. Plasma changes of mannose during 120 min from base line ($M_{120}-M_0$) were significantly different among groups (NGT: -16.7±8.2; IGT: -9.0±9.8; DM: -1.4±9.3 µM, $P<0.001$ ANOVA). Simple regression analysis revealed that $M_{120}-M_0$ was not significantly associated with Im, but was significantly associated with IGI ($R=-0.282$, $P=0.014$), QUICKI ($R=0.237$, $P=0.041$), Matsuda index ($R=0.254$, $P=0.027$), and Gm ($R=0.544$, $P<0.001$).

Conclusion: The plasma mannose level was decreased after glucose load in normal glucose tolerance, but was not decreased in diabetes. The decrease in plasma mannose level after glucose load was associated with glucose tolerance, represented by the mean glucose level after glucose load. Moreover, it was associated with indices of insulin secretion and insulin sensitivity. These results support the possibility of the plasma mannose level as an indicator of glycogenolysis in human.

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Analysis of pancreatic response after oral glucose tolerance test in adults with cystic fibrosisI. Sánchez-López¹, M.A. Sampedro-Núñez¹, S. Campos¹, A. Vicuña¹, M. Zelada¹, M.A. Maíllo¹, A. Mossé¹, A.M. Ramos-Leví¹, R.M. Girón², M. Marazuela¹, A. Arranz¹;¹Endocrinology,²Pulmonary Medicine, Hospital Universitario de La Princesa, Madrid, Spain.

Background and aims: Abnormalities in carbohydrate metabolism are particularly relevant in adults with cystic fibrosis (CF) because of their high prevalence (about 75 percent), and impact on morbidity and mortality in these patients. Oral glucose tolerance test (OGTT) provides important information about pancreatic secretion in these subjects. The aim of this study is to analyze baseline and pancreatic response along OGTT in adults with CF.

Materials and methods: Retrospective study based on the performance of OGTT in 37 adult patients with CF followed in our hospital. Glucose, C-

Peptide and insulin were measured at fasting and at 30, 60, 90 and 120 minutes after a 75 grams glucose load. According to the results, patients were classified into: normal glucose tolerance (NGT), impaired glucose tolerance (IGT), indeterminate glycemia (INDET) and cystic fibrosis-related diabetes (CFRD). The following insulin secretion indexes were determined: HOMA-β, insulinogenic index (II) and first phase-OGTT (FIOGTT). Areas under the curve (AUC) were calculated for each group, and variance for the different variables was analyzed. Statistical analysis was performed using SPSS for Windows, version 15.0.

Results: 37 patients. OGTT **Results:** 35.1% NGT; 32.4% IGT; 13.5% INDET; 18.9% CFRD. Significant differences ($p<0.05$) between groups were obtained at all times of the glucose curve except at fasting, and at 120 minutes for the ratio glucose/insulin. A greater glucose AUC in CFRD patients and a greater overall insulin and C-Peptide secretion in NGT and INDET groups were observed. In early stages of the OGTT, lower values of insulin and HOMA-β, II and FIOGTT indexes were observed in INDET group ($p<0.05$). However, from time 60 min onwards, an increased insulin response in INDET group was observed compared to the rest.

Conclusion: OGTT shows a progressive deterioration of insulin secretion in prediabetic states. INDET and IGT subjects have a failure of the early response and a delayed pancreatic secretion as a preliminary step to the development of CFRD.

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Relationship of whole-body fuel oxidative partitioning with insulin secretion and beta cell function in non-diabetic humansJ.E. Galgani¹, M.L. Mizgier¹, A. Mari², E. Ravussin³;¹Pontificia Universidad Católica de Chile, Santiago, Chile,²Istituto di Ingegneria Biomedica, Padova, Italy,³Pennington Biomedical Research Center, Baton Rouge, USA.

Background and aims: It is commonly reported that whole-body insulin sensitivity is inversely related with glucose-stimulated insulin secretion. This finding suggests a putative crosstalk between peripheral organs and pancreas. Such organ crosstalk could be mediated by changes in glucose flux (i.e. uptake, oxidation or storage) in insulin-sensitive tissues, which may lead to the release of a circulating factor driving insulin secretion. To gain insight on such a metabolic feedback loop, we related fasting, postprandial and 24-hour whole-body non-protein respiratory quotient (npRQ), an index of fuel oxidative partitioning (i.e. carbohydrate relative to fat oxidation), with insulin secretion and beta cell function in non-diabetic individuals.

Materials and methods: Thirty non-diabetic participants (15/15 males/females; 35±12 [SD] years old; 27±4 kg/m²) were studied over three days. On day 1, npRQ was determined under fasting and postprandial (75-gram oral glucose tolerance test, OGTT) conditions. On day 2, participants spent 24 hours in a respiratory chamber while fed with a standard, isoenergetic mixed diet. On day 3, insulin sensitivity was assessed by a 2-hour euglycemic-hyperinsulinemic clamp. Insulin secretion was estimated by deconvolution of serum C-peptide concentration (fasting and postprandial) or 24-hour urinary C-peptide excretion (corrected for energy intake). In addition, beta cell function parameters including rate sensitivity (indicative of first-phase glucose-induced insulin response) and glucose sensitivity (slope of the glucose concentration vs. insulin secretion relationship) were calculated by mathematical modelling.

Results: Insulin secretion estimated over 24 hours from urinary C-peptide excretion was directly related with fasting ($r=0.64$; $p<0.001$) and glucose-stimulated ($r=0.66$; $p<0.001$) insulin secretion. The classical inverse association between insulin sensitivity (by clamp) and insulin secretion was also confirmed over 24 hours ($r=-0.62$; $p<0.01$), in response to an OGTT ($r=-0.50$; $p<0.01$) and overnight fasting ($r=-0.40$; $p<0.05$). Interestingly, insulin secretion (from urinary C-peptide excretion) was inversely correlated with 24-hour npRQ ($r=-0.61$; $p=0.001$), even after controlling for insulin sensitivity and energy balance ($r=-0.52$; $p=0.01$). However, fasting or glucose-stimulated npRQ did not correlate with insulin secretion rate (from serum C-peptide). Fasting npRQ correlated directly with rate sensitivity ($r=0.40$; $p<0.05$), while the association with glucose sensitivity was borderline ($r=0.34$; $p=0.08$).

Conclusion: Fuel oxidative partitioning determined over a day appears to influence 24-hour insulin secretion, although such association did not hold when a shorter period was considered. In addition, the first-phase glucose-induced insulin response (i.e. rate sensitivity) may be sensitive to the level of fasting fuel oxidative partitioning. These findings lend support to the hypothesis that a crosstalk between peripheral tissues and pancreas plays a role on insulin secretion. Intervention studies targeting peripheral (e.g. skeletal

muscle) carbohydrate oxidation and its effect on insulin secretion deserve further research.

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Role of parasympathetic activity in insulin secretion in metabolically healthy obese patients

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Background and aims: Cardiac parasympathetic activity is often altered in obese non-diabetic patients, even in the metabolic healthy obese individuals. We here hypothesized that the persistence of a good parasympathetic activity could contribute to a better insulin secretion in the presence of insulin resistance.

Materials and methods: We recruited 47 obese patients (BMI 42.3 ± 6.5 kg/m², 35.5 ± 12.7 years). Among them 31 with only one criterion of the metabolic syndrome (fasting hyperglycemia in only one patient) in addition to a large waist circumference was considered as metabolically healthy. Cardiac parasympathetic activity (HF-HR) and vascular sympathetic activity (LF-SBP) were evaluated by spectral analysis of heart rate (HR) and systolic blood pressure (SBP) variations (Finapres®), respectively. An oral glucose tolerance test was performed. Two indexes of insulin resistance (Matsuda and HOMA-IR), and composite indexes of insulin secretion (insulinogenic index = $\text{IGI} = \Delta \text{insulinemia}(\text{T0-T30}) / \Delta \text{glycemia}(\text{T0-T30})$; and oral disposition index = $\text{ODI} = \text{IGI} / \text{insulinemia}$) were calculated.

Results: Patients with a lower parasympathetic activity (HF-HR < median value: 2.61) had a lower Matsuda index ($p = 0.012$), and ODI ($p < 0.03$), higher plasma glucose levels at 90 and 120 min after glucose challenge (p median value) while age and BMI were similar. In metabolically healthy patients, but not in patients with more metabolic criteria, the same results were found, noticeably with plasma glucose during OGTT (at 60, 90, 120, 150, 180 min; $p = 0.007$ to 0.058) and LF-SBP ($p = 0.006$) higher in those with HF-HR < median, and a significant correlation of ODI with HF-HR ($r = 0.464$, $p = 0.008$).

Conclusion: These results strongly suggest that vagal activity contributes to enhance insulin secretion in insulin resistant obese individuals. In metabolically healthy obese patients, low vagal activity together with a predominant sympathetic activity is associated with a more marked insulin resistance and reduced compensatory insulin secretion, leading to glucose disorders.

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Impact of serum triglyceride and HDL-cholesterol levels on early-phase insulin secretion in prediabetic subjects

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Background and aims: β cell dysfunction plays a fundamental role in the onset and progression of type 2 diabetes (T2DM), and the loss of early-phase insulin secretion is thought to be an early marker of T2DM. Recent studies have shown that increased triglycerides (TG) and decreased HDL-cholesterol (HDL-C) levels are risks for the onset of T2DM. However, in subjects with prediabetes (impaired fasting glucose, impaired glucose tolerance and the two combined), the association between lipid profiles and insulin secretion has not been well studied. The aim of this study was to investigate the relationship between serum TG and HDL-C levels and early-phase insulin secretion following a 75-g oral glucose tolerance test (OGTT) in prediabetic subjects.

Materials and methods: We evaluated 663 prediabetic subjects (322 males and 341 females; age, 52 ± 8 years; BMI, 23.8 ± 3.1) who were not taking lipid-lowering agents. Prediabetes was defined as fasting plasma glucose of 5.6–6.9 mmol/L or 2h post-challenge plasma glucose of 7.8–11.0 mmol/L. Insulin secretion was estimated by the insulinogenic index (IGI) [$\Delta \text{insulin} / \Delta \text{glucose} (30 \text{ min} - 0 \text{ min})$] and disposition index (DI; $\text{IGI} / \text{HOMA-IR}$), which is an adjusted measure of β cell function that accounts for variations in insulin sensitivity. The relationship between continuous variables was examined by Pearson's linear regression analysis. A stepwise multiple linear regression analyses was performed to assess independent relationships between DI and the clinical variables including age, sex, BMI, blood pressure, uric acid, total cholesterol (TC), TG, HDL, LDL-cholesterol (LDL-C), HbA1c, eGFR and

smoking status. Because of collinearity between TG and HDL-C, two regression models were performed, which took into account each of these variables. **Results:** Pearson's linear regression analysis revealed that IGI and DI were positively correlated with HDL-C levels ($r = 0.264$, $P = 0.004$ and $r = 0.278$, $P = 0.002$, respectively). Moreover, the indices were negatively correlated with the log-transformed TG ($r = -0.190$, $P = 0.006$ and $r = -0.133$, $P = 0.004$, respectively). However, TC and LDL-C were not associated with indices of insulin secretion. Multivariate linear regression analyses revealed that DI was independently and positively correlated with HDL-C ($\beta = 0.149$, $P = 0.004$) and negatively with log-transformed TG ($\beta = -0.249$, $P = 0.004$).

Conclusion: Our results provide evidence that, in prediabetic subjects, lower HDL-C and higher TG levels are associated with dysfunctional early-phase insulin secretion. Thus, more attention should be placed on serum HDL-C and TG levels in prediabetic subjects because of their potential progression to T2DM.

PS 028 Insulin secretion in diabetes

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Most people with long duration of type 1 diabetes are insulin microsecretors and produce their own endogenous insulin

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Background and aims: Ultrasensitive assays that can detect C-peptide under 5 pmol/L allow detection of very low levels of c-peptide. We aimed to use urine c-peptide creatinine ratio (UCPCR) to assess endogenous insulin in a large cross-sectional population-based study of patients with Type 1 diabetes (T1D).

Materials and methods: We recruited 944 patients from primary and secondary care in 2 UK centres. All diagnosed under 30 years, duration >5 years, clinical diagnosis of T1D. Median (IQR) age of diagnosis 11 (6–17) y, duration 18 (11–26) y, HbA1c 8.7 (7.9–9.8)%, insulin dose 0.78 (0.60–0.97) u/kg/24hr, and BMI 25.6 (23.3–28.6) Kg/m². All provided a home post-meal UCPCR.

Results: 81% (790/944) had detectable endogenous production (median (IQR) UCPCR 0.012 (0.004–0.038) nmol/mmol). Most had very low, historically undetectable, levels (492/944, 53%, UCPCR >0.001–0.03 nmol/mmol). 8% had C-peptide levels above the DCCT cut off of significant endogenous insulin. Absolute UCPCR levels fell with duration but the proportion with detectable UCPCR never fell below 73% (maximum duration 47 years). Age of diagnosis and duration independent predictors of C-peptide in multivariate modelling.

Conclusion: The majority of patients with long duration T1D are insulin microsecretors and have detectable urine c-peptide. Some rare individuals with T1D maintain higher levels of endogenous insulin for many years after diagnosis of diabetes. The fact that some beta cells remain in most with long-standing T1D may reflect escape from immune attack, or beta cell regeneration. Understanding this may lead to a better understanding of pathogenesis in T1D and open new possibilities for treatment.

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Presence and titer of GAD antibodies are determinants of beta cell function in patients with newly diagnosed type 2 diabetes mellitus: further insights in the metabolic phenotype of LADA patients

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Background and aims: Latent Autoimmune Diabetes in Adults (LADA) is a metabolic disorder at the crossroad between type 1 (T1DM) and type 2 diabetes (T2DM). Aim of our study was to carefully assess beta cell function and insulin sensitivity in patients with LADA, in comparison to patients with either type 2 diabetes clinically undistinguishable from LADA or typical type 2 diabetes.

Materials and methods: In 35 (M/F=19/16) patients (mean±SEM: age 57.4±1.6 years, BMI 27.5±0.9 kg/m²) with newly diagnosed LADA were compared to 35 patients with newly diagnosed T2DM matched for age, gender, BMI and HbA1c (LADA-like). The latter group was extracted from the database of the Verona Newly Diagnosed Type 2 diabetes (VNDS; N=589 GADA-negative patients). The rest of VNDS patients herein represent typical T2DM. LADA patients were further divided in two groups according to GADA levels (median 4 kU/L): low GAD-LADA (GADA below 4kU/L) and high GAD-LADA (GADA above 4kU/L). In all patients we performed on separate days: 1. prolonged (5-hours) frequently sampled OGTT to assess derivative control (DC) and proportional control (PC) of beta cell function by state of art mathematical modeling of glucose and C-peptide curves; 2. standard euglycemic insulin clamp to assess insulin sensitivity (SI).

Results: SI was not statistically different (p<0.12) in LADA-like and in LADA patients, but in the latter was higher (+28%) than in VNDS (812±66 vs 635±16 μmol.min-1.m-2 BSA, respectively; p=0.01). The DC of beta cell function was impaired in LADA compared to LADA-like (p<0.01) and to VNDS (p<0.05). The PC in LADA was similar to LADA-like (p=0.42), but it was reduced when compared to VNDS (p<0.03). High GAD- and low GAD-LADA had similar SI, but the former had worse PC of beta cell function than the latter (p<0.01).

Conclusion: Patients with newly diagnosed LADA display more severe defects in beta cell function even when compared to LADA-like patients with newly diagnosed T2DM; furthermore, the higher the GADA titer, the worse is beta cell dysfunction. These data may be of help in optimizing metabolic therapy and in refining metabolic prognosis of these patients.

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Influence of the human herpes virus 8 infection on beta cell function and insulin sensitivity in ketosis prone diabetes

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Background and aims: Ketosis Prone diabetes (KPD), an atypical diabetes with ketosis at onset and absence of β-cell autoantibodies is characterized after the ketotic episode by long-term non-insulin dependence (remission) for a majority of patients. Human herpes virus 8 (HHV-8) infection has been associated with KPD in patients of sub-Saharan African origin. Our objective was to investigate whether HHV-8 infection impacts on insulin secretion and/or insulin sensitivity in KPD at different stages of the disease.

Materials and methods: We compared patients with (KPD+) and without (KPD-) HHV-8 infection in 3 different groups. In group 1, 11 KPD+ and 7 KPD- in remission (HbA1c level <7.0% at least 3 months apart after withdrawal of exogenous insulin treatment) underwent an OGTT, a glucose ramping + arginine test and a hyperinsulinemic euglycaemic clamp. Group 2 consisted of 17 KPD+ and 11 KPD- explored one week after ketosis resolution by C-peptide measurement in response to IV glucagon (delta C-peptide) and by the short insulin tolerance test (ITT). In group 3, we enrolled 40 KPD+ and 59 KPD- hospitalized for treatment adjustment because of elevated A1c. All KPD underwent delta C-peptide at 2 different occasions (V1 and V2). V1 was made 3.1±2.5 [mean±SD] years after the diagnosis of diabetes and V2 conducted 3.6±5.4 years after V1. Detection of HHV-8 antibodies was performed using 3 techniques, one for detection of antibodies against lytic antigens, a second against latent nuclear antigens and the last detecting the HHV-8 capsid protein.

Results: In each group, KPD- and KPD+ were comparable in terms of age, age at diabetes diagnosis, duration of diabetes, duration of remission (group 1), BMI and HbA1c (group 1, group 2). In group 1, the insulinogenic index and insulin secretion rates were higher in KPD+ compared to KPD-, whereas insulin secretion in response to arginine and insulin sensitivity were similar. In group 2, delta C-peptide was up to 3-fold higher in KPD+ compared to KPD- while the glucose disappearance rate K_{ITT} were similar between the 2 groups. In group 3, A1c levels in KPD+ were lower than KPD- and delta C-peptide was higher in KPD+ compared to KPD- at V1 and V2.

Conclusion: In conclusion, either in remission, shortly after ketosis resolution or during long term follow-up, latent HHV-8 infection is associated with better insulin secretion in response to glucose in patients of sub-Saharan African origin with KPD. Mechanisms by which latent HHV-8 infection impact on β -cell functions need to be unraveled.

		KPD+	KPD-	*p
Insulin secretion	Insulinogenic index(μ U/nmol) (OGTT)	6.12 \pm 4.06	2.72 \pm 1.65	0.05*
	Insulin secretion rate (pmol/kg/min) (Glucose ramping 10 mg/kg/min)	8.1 \pm 3.6	5.7 \pm 4.7	0.043*
	Arginine test: Acute insulin response (μ U/mL)	407.2 \pm 274.07	207.36 \pm 138.46	0.2
	Insulin sensitivity (Clamp)			
	Glucose disposal rate (mg/kg free fat/min)	6.56 \pm 3.2	8.08 \pm 2.37	0.246
Insulin secretion (Glucagon test)	Baseline C-peptide(ng/mL)	1.62 \pm 1.18	0.46 \pm 0.22	0.001*
	Delta C-peptide(ng/mL)	2.42 \pm 2.29	0.72 \pm 0.45	0.004*
Insulin sensitivity (ITT)	K _{ITT} (%·min ⁻¹)	1.87 \pm 1.17	1.24 \pm 0.86	0.13
V1 HbA1c (%)		11.1 \pm 3.8	12.5 \pm 3.0	0.041*
Insulin secretion Visit 1	V1 Baseline C-peptide(nmol/L)	1.21 \pm 1.16	0.51 \pm 0.45	0.01*
	V1 Delta C-peptide(nmol/L)	1.6 \pm 2.0	0.51 \pm 0.48	0.0001*
V2 HbA1c (%)		8.2 \pm 2.2	9.4 \pm 2.8	0.11
Insulin secretion Visit 2	V2 Baseline C-peptide(nmol/L)	1.34 \pm 1.22	0.68 \pm 0.58	0.03*
	V2 Delta C-peptide(nmol/L)	1.63 \pm 1.89	0.79 \pm 0.83	0.09

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Correlation between the indices for glycaemic variability and 24-hour urinary C-peptide values in patients with type 1 diabetes mellitus as assessed by CGM

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Background and aims: Loss of pancreatic cell function, abnormal secretion of the counter-regulatory hormones, inappropriate treatment, insulin resistance, malingering/manipulation, concomitant diseases such as infection and some other endocrine disease, and mental/somatic stress are reported to be among the factors that tend to make glycemic control difficult in patients with type 1 diabetes. First and foremost of these are, however, loss of pancreatic β cell function and abnormal secretion of the counter-regulatory hormones. Therefore, stable glycemic control becomes progressively lost with the loss of pancreatic β cell function. While, generally, it often proves extremely difficult to achieve glycemic control in type 1 diabetes, not all patients with type 1 diabetes exhibit unstable diabetes and a subset of patients exhibit stable glycemic control. In the latter patients, pancreatic β cell function is shown to be preserved to a certain extent, thus contributing to their stable glycemic control. However, to date, there are very few studies in which residual pancreatic β cell function was examined for correlation with glycemic variation evaluated by using Continuous Glucose Monitoring(CGM) that allows glucose levels to be measured on a continuous basis.

Materials and methods: Of all patients being treated with intensive insulin therapy (excluding Continuous Subcutaneous Insulin Infusion) and monitored by CGM, a total of 59 patients with type 1 diabetes (males/females, 20/39) with detectable urinary C-peptide immunoreactivity (U-CPR) from 24-hour samples were included in the study. Values measured for continuous 24 hours and free from measurement errors were used for current analysis. U-CPR values were used as an index for insulin-secretory capacity and the SD of 24hour-glucose concentration was used as an index for stability of glycemic control. The subjects' U-CPR values were examined for correlation with their duration of disease, BMI, and HbA1c values, as well as the SD of their glucose concentration. Statistical analyses were performed by using SPSS 17.0. Spearman's rank sum correlation coefficients were used to test for correlation between the variables examined.

Results: All data are shown as median and interquartile range. The subjects' median U-CPR value was 1.4 (0.9-6.9) μ g/day, their median age 39 (32-58) years, duration of disease 11 (6-22) years, BMI 21.2 (19.4-23.2) kg/m², HbA1c value 8.0 (7.1-9.4)%, their total insulin dose 36 (28-48) units, and their basal

insulin ratio(basal insulin dose/total insulin dose) 44.4 (37.5-54.3)%. A significant negative correlation was noted between their U-CPR values and the SD of their glucose concentration ($r = -0.305$; $P = 0.019$), as well as between their U-CPR values and their duration of disease ($r = -0.353$; $P = 0.006$), and between their U-CPR values and their basal insulin ratio ($r = -0.268$; $P = 0.040$). However, there was no significant correlation between their U-CPR values and their age, BMI, HbA1c values or total insulin units.

Conclusion: Our study demonstrated that the lower the U-CPR values in patients with type 1 diabetes, the greater their glycemic variability and that the longer their duration of disease, the lower the U-CPR values, suggesting that U-CPR assessment may have a key role in predicting glycemic variations in patients with type 1 diabetes.

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Factors affecting glycaemic variability in patients with type 2 diabetes

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Background and aims: It is not widely known about factors determining glucose fluctuations in diabetic patients. Also, the role of glycemic variability in the development of cardiovascular diseases remains controversial. We investigated the relationship between indices of glycemic variability, cardiovascular (CV) risk factors, and clinical and laboratory variables in type 2 diabetic patients.

Materials and methods: We enrolled 236 type 2 diabetic patients which performed a 7-point SMBG more than once during each month for 3 consecutive months. From their SMBG data, glycemic variability indices (standard deviation (SD) and M-value) were calculated monthly. HbA1c was measured on the last day of the third month. BMI, waist circumference (WC), blood pressures (BP), duration of diabetes, hsCRP, fibrinogen, ALT, GGT, creatinine, uric acid, total cholesterol, triglyceride (TG), HDL-C, LDL-C, urine albumin:creatinine ratio (UACR) and ankle-brachial pressure index (ABI) were assessed. Treatment stage of diabetes was defined based on the number of oral hypoglycemic agents and the administration of insulin. The 10-year risk for CVD was calculated using 2013 ACC/AHA Prevention Guidelines Atherosclerotic Cardiovascular Disease (ASCVD) Risk Estimator.

Results: The SD was significantly correlated with duration of diabetes ($r=0.291$; $p<0.001$) and UACR ($r=0.144$; $p=0.028$), but not with BMI, WC, BP, hsCRP, fibrinogen, ALT, GGT, creatinine, uric acid, lipid profile and ABI. The M-value was correlated with duration of diabetes ($r=0.301$; $p<0.001$), UACR ($r=0.210$; $p=0.001$), BMI ($r=0.129$; $p=0.049$), WC ($r=0.163$; $p=0.013$) and TG ($r=0.181$; $p=0.005$), but not with other parameters. Using hierarchical regression analysis to adjust for HbA1c and other covariates, only diabetes duration remained independent correlate of the SD ($\beta=0.200$, $p<0.01$) and M-value ($\beta=0.154$; $p<0.01$). CV risk factors failed to maintain its independent association. The patients were divided into three groups according to diabetes treatment stage and 10-year ASCVD risk, respectively. SD and M-value showed significant differences according to increasing treatment stage (pANOVA<0.001; pTREND<0.001 and pANOVA<0.001; pTREND<0.001, respectively), but not increasing 10-year ASCVD risk (pANOVA=0.707; pTREND=0.518 and pANOVA=0.472; pTREND=0.780, respectively).

Conclusion: In this study, duration of diabetes rather than CV risk factors was an independent variable of indices of glycemic variability. And the indices were positively correlated to treatment stage of diabetes more than 10-year ASCVD risk. These findings suggest that glycemic variability is largely determined by β -cell function which deteriorates with increasing duration of diabetes, but not cardiovascular complication.

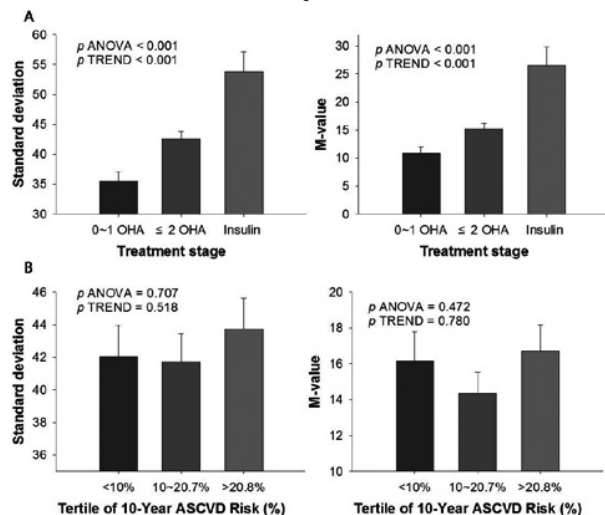


Figure 1. Plots of glycemic variability indices according to stage of diabetic treatment (A) and 10 year atherosclerotic cardiovascular disease (ASCVD) risk (B).

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Loss of glucose sensitivity and islet neogenesis predict the occurrence of diabetes after acute beta cell reduction

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Background and aims: Progressive deterioration in β cell function and decrease in β cell mass represent the main mechanisms involved in type 2 diabetes. To investigate if the deterioration of the β cell function corresponds to a loss of β cell mass, we performed oral glucose tolerance tests (OGTT), hyperglycemic clamps (HC) and followed by arginine stimulation in 16 patients undergoing pancreatoduodenectomy (PD), pre- and post-surgery. To further explore whether Islet features could be justified by in vivo beta cell function, we explored neogenesis from duct cells, islet size and trans-differentiation of a cells to β cells.

Materials and methods: Based on post-surgery OGTT, subjects were divided into 3 groups of glucose tolerance: normal (NGT, n=5), impaired (IGT, n=4) or diabetes (DM, n=7) (8 F/8 M, 51 \pm 15 yrs.). To evaluate β cell function, β cell glucose sensitivity (GS) during HC was calculated as the ratio of insulin secretion and glucose increments. During surgery, pancreas samples were collected for IHC for glucagon, insulin and somatostatin+ cells to assess islet morphology. Ductal cells were stained by CK19.

Results: Before surgery, Arginine-stimulated Insulin Secretion (AIS) was similar across groups, whereas GS was lower in IGT and DM as compared with NGT subjects (62.9 \pm 23.1 and 45.5 \pm 11.2 vs 90.6 \pm 18.7 pmol \cdot min $^{-1}\cdot$ m $^{-2}$, respectively). Following 50% PD, GS decreased in all patients ($p<0.01$ for all groups), but the reduction was greater in DM compared to IGT and NGT patients (Δ GS: NGT -0.20 \pm 0.19 vs. IGT -0.27 \pm 0.11 vs. DM 0.37 \pm 0.08; $p<0.003$). A similarly scaled reduction was observed in Δ AIS (NGT -0.38 \pm 0.13 vs. IGT -0.76 \pm 0.06 vs. DM -0.90 \pm 0.04; $p<0.01$) and in 2nd phase insulin secretion. IHC demonstrated an increase in islet size, insulin+ CK19+ cells ($p<0.05$) and scattered islets (<8 cells) ($p=0.01$) in DM patients, as compared with NGT and IGT.

Conclusion: In this study, loss of GS was the most reliable parameter for predicting the appearance of diabetes after 50% PD. Increased islet size and neogenesis could be compensatory mechanisms to cope with reduced function (GS) and to preserve β cell mass, at least as estimated by AIS. However, increased islet size does not seem to be able to compensate for the loss of glucose sensitivity.

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Serum bilirubin concentrations are positively associated with serum C-peptide levels in patients with type 2 diabetes

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Background and aims: Bilirubin, the natural end product of heme metabolism, has been recognized as a potent endogenous antioxidant. Several clinical studies have demonstrated the inverse correlation between serum bilirubin levels and the risk of diabetes. However, the relationship between serum bilirubin levels and serum C-peptide levels in patients with type 2 diabetes has not been fully understood. The aim of the study was to investigate the relationship between physiological serum total bilirubin concentrations and serum C-peptide levels in Korean patients with type 2 diabetes.

Materials and methods: A total of 588 patients with type 2 diabetes (M: 337, F: 251, mean age 56.4 \pm 13.6 years) were investigated in this cross-sectional study. All patients were allowed to eat a standardized meal. Fasting C-peptide level, 2-hour postprandial C-peptide level, and Δ C-peptide (postprandial C-peptide minus fasting C-peptide) level were measured in all patients.

Results: Fasting C-peptide level, postprandial C-peptide level, and Δ C-peptide level tended to be higher in patients with increased bilirubin concentrations. Partial correlation analysis showed that serum bilirubin levels were significantly correlated with fasting C-peptide level ($r=0.159$, $P<0.001$), postprandial C-peptide level ($r=0.209$, $P<0.001$), and Δ C-peptide level ($r=0.186$, $P<0.001$) after adjustment for other covariates. In the multivariate model, the association between serum bilirubin concentrations and serum C-peptide levels remained significant, after adjustment for confounding factors including age, gender, familial diabetes, hypertension, hyperlipidemia, body mass index, glycated hemoglobin, duration of diabetes, and associated liver function tests (fasting C-peptide level: $\beta=0.083$, $P=0.041$; postprandial C-peptide level: $\beta=0.106$, $P=0.005$; Δ C-peptide level: $\beta=0.096$, $P=0.015$, respectively).

Conclusion: Serum bilirubin concentrations within the physiologic range were positively associated with serum C-peptide levels in patients with type 2 diabetes.

Table 1. Multivariate linear regression analysis with serum C-peptide concentration as a dependent variable

	Partial regression coefficient (SE)	Standard partial regression coefficient	P-value
Fasting C-peptide level*			
Total bilirubin	0.065 (0.032)	0.083	0.041
BMI	0.008 (0.002)	0.186	<0.001
Duration of diabetes*	-0.056 (0.017)	-0.141	0.001
HbA _{1c}	-0.019 (0.003)	-0.252	<0.001
R ² (adjusted R ²)		0.183 (0.168)	
Postprandial C-peptide*			
Total bilirubin	0.100 (0.035)	0.106	0.005
BMI	0.008 (0.002)	0.143	<0.001
Duration of diabetes*	-0.087 (0.019)	-0.180	<0.001
HbA _{1c}	-0.043 (0.004)	-0.472	<0.001
R ² (adjusted R ²)		0.333 (0.321)	
Δ C-peptide*			
Total bilirubin	0.110 (0.045)	0.096	0.015
BMI	0.004 (0.003)	0.064	0.135
Duration of diabetes*	-0.097 (0.024)	-0.167	<0.001
HbA _{1c}	-0.049 (0.005)	-0.436	<0.001
R ² (adjusted R ²)		0.249 (0.235)	

* Data were log-transformed before analysis. To convert μ mol/L to mg/dL, divided by 17.1.

Adjusted for age, gender, ALT*, family history of diabetes, free fatty acid*, hypertension, and hyperlipidemia.

BMI, body mass index; HbA_{1c}, glycated hemoglobin

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Improved beta cell and disposition function leads to reduction in daily insulin dosage over time in patients with type 2 diabetes treated by long-term CSII therapy

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Background and aims: We observed the daily insulin dosage required to maintain normoglycemia decreased over time during long-term continuous insulin infusion (CSII) therapy in patients with type 2 diabetes. To see if the decreased daily insulin dosage is related to changes in beta cell function and insulin resistance during CSII therapy, we examined changes in C-peptidogenic Index (CI), Matzda Index (MI), and disposition Index (DI; product of CI and MI) for 1 year.

Materials and methods: Two hundred seventeen patients with type 2 diabetes (age, 59.1 ± 11.1 years; male, 113; female, 104; duration, 12.6 ± 10.2 years; body mass index, 23.7 ± 3.7 kg/m²; HbA1c 8.9 ± 2.1 %) were treated by CSII. Blood samplings were performed at baseline and 6 months and 1 year after CSII therapy (after overnight fasting and 2 hours after ingestion of 500 kcal-mixed meal).

Results: After 1 year of CSII therapy, HbA1c decreased from 8.9 ± 2.1 to 7.3 ± 1.7 % ($p < 0.001$), serum C-peptide level increased from 4.8 ± 2.8 to 6.0 ± 2.9 ng/ml ($p < 0.001$), and CI increased from 0.021 ± 0.018 to 0.029 ± 0.017 ($p < 0.001$) at 2 hours after meal ingestion. DI increased from 0.060 ± 0.059 to 0.069 ± 0.045 ($p < 0.003$). Daily insulin dosage decreased from 71.7 ± 34.4 to 46.3 ± 31.1 IU/day ($p < 0.001$). This decreased daily insulin dosage was associated with the increased value of CI, DI and MI significantly by multiple regression analysis ($p < 0.05$).

Conclusion: The resolution of glucotoxicity through long-term CSII therapy may contribute to restoration of beta cell function and disposition capability in type 2 diabetic patients. It is suggested that the daily insulin requirement decreased due to improved beta cell function and disposition capability during long-term CSII therapy in type 2 diabetic patients.

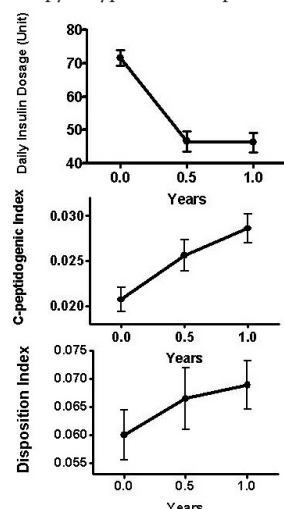


Figure 1. Change of daily insulin dosage, C-peptidogenic index and disposition index during one year of CSII therapy. All data points are different significantly from baseline ($p < 0.001$).

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Synergistic beta cell effect of macronutrients in a mixed meal is preserved in drug-naïve subjects with type 2 diabetes

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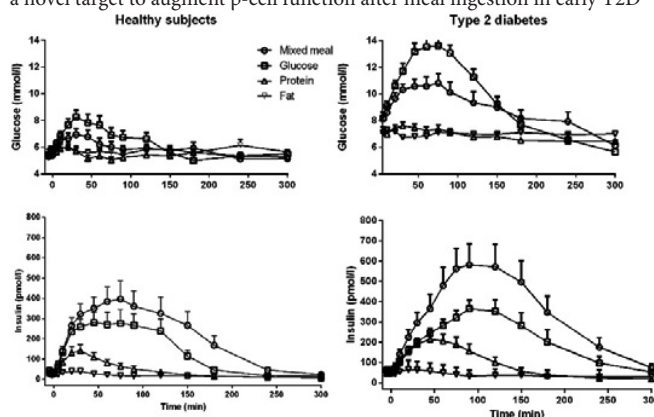
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Background and aims: The macronutrient composition of a meal is fundamental for glycemic control through their synergistic stimulation of insulin secretion in healthy subjects. Since the relevance of this synergistic effect for development of type 2 diabetes (T2D) is unknown, we have explored it in drug-naïve well-controlled subjects with T2D compared to age- and BMI-matched healthy subjects.

Materials and methods: After an overnight fast, eighteen (13M,5F) well-controlled drug-naïve T2D subjects (mean age 64 yrs, BMI 27.2 kg/m², HbA1c 44 mmol/mol=6.0%) and eighteen (11M,7F) healthy subjects (mean age 63 yrs, BMI 25.3 kg/m²) underwent four tests in random order ingesting either a liquid mixed meal (550kcal; 60% carbohydrate, 20% protein and 20% fat) or each of the individual macronutrients: glucose (83g=330kcal) protein (whey protein, 30g=110kcal) or fat (sunflower and rapeseed oil, 12g=110kcal); all tests 350ml; samples were taken for analysis of glucose, insulin and intact GLP-1 (iGLP-1); their Suprabasal (sb) and total (t) 120 min areas under the curve (AUC) were calculated. β -cell function estimated as insulinogenic index [IGI=sb AUCinsulin/sb AUCglucose] and oral glucose insulin sensitivity (OGIS) were calculated from the glucose and insulin concentration.

Results: In healthy subjects, mixed meal (which included all three macronutrients) augmented insulin levels and β -cell function compared to glucose alone which resulted in lower postchallenge glycemia (IGI 49 ± 7.2 vs. 33.8 ± 5.5 pmol/mmol, $p=0.0009$): tAUCglucose: 776 ± 36 vs 890 ± 52 pmol/l min, $p=0.009$ with no significant difference in iGLP-1 levels (tAUCGLP-1 802 ± 118 vs 926 ± 123 pmol/l min, $p=0.5$). T2D subjects showed exactly the same augmenting effects on β -cell function of mixed meal vs. glucose alone (IGI: 42.6 ± 7 vs. 22.5 ± 5.5 pmol/mmol $p=0.007$) and tAUCglucose: 1231 ± 63 vs 1484 ± 18.2 mmol/l min, $p<0.0001$ with, again, no significant difference in iGLP-1 levels (tAUCGLP-1 933.6 ± 156 vs 939 ± 134 pmol/l min, $p=0.9$). In fact, the relative increase of IGI by mixed meal compared to glucose alone was well preserved in T2D (19.9 ± 5.4 vs 15.8 ± 3.4 pmol/mmol, $p=0.5$). OGIS was higher in healthy subjects than in T2D after both mixed meal ($398 \pm 15,304 \pm 15$ ml/min/m², $p=0.0005$) and glucose ($407 \pm 14,324 \pm 12$ ml/min/m², $p=0.003$).

Conclusion: Adding protein and fat to glucose in a mixed meal results in increased insulin levels due to augmented β -cell function which prevents hyperglycemia when compared to ingestion of glucose alone. This synergistic effect of protein and fat on β -cell function, being not associated with augmented iGLP-1 levels, is well preserved in early stages of T2D pinpointing that a synergistic macronutrient effect may attempt to rescue the failing β -cell response to glucose. The detailed mechanism of this synergy may offer a novel target to augment β -cell function after meal ingestion in early T2D



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PS 029 Gut hormones

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Impact of Roux-en-Y gastric bypass on distribution of enteroendocrine cells and their gene expression in obese patients with type 2 diabetes and non-diabetic controls

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Background and aims: RYGB is a weight-reducing surgical procedure, which changes macroscopic gut anatomy and the route of ingested nutrients and secretory products through the gastrointestinal tract, resulting in weight loss and improved glucose homeostasis in the majority of subjects. The aim of this study was to describe the impact of Roux-en-Y gastric bypass (RYGB) on the distribution of small intestinal enteroendocrine cells and the expression of their hormonal products in obese patients with type 2 diabetes and non-diabetic controls.

Materials and methods: Twelve obese subjects with type 2 diabetes and 11 age and BMI matched controls underwent RYGB and enteroscopy at least 4 months later. Mucosa biopsies from both procedures were immunohistochemically stained for cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) and gene expression analyses were performed looking at ghrelin (GHL), CCK, secretin, GIP, GLP-1, PYY, neurotensin and farnesoid X receptor (FXR).

Results: Mean time (\pm SEM) of enteroscopy after RYGB was 10.4 \pm 0.8 months. Immunohistochemically there was an increase in the density of cells staining positive for CCK, GIP, GLP-1 and PYY (controls only) after vs. before RYGB. The gene expression of GHL, secretin and GIP mRNA was down-regulated after RYGB. CCK, PYY, neurotensin and FXR mRNA gene expression was unaltered after RYGB whereas GLP-1 mRNA was up-regulated in both groups.

Conclusion: Numerous alterations in the distribution of enteroendocrine cells and their expression of hormonal products is seen after RYGB. These changes may play an important role in the metabolic improvements seen after RYGB.

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Insulin-like peptide 5 is an orexigenic gastro-intestinal hormone secreted from colonic L-cells

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Background and aims: Intestinal hormones secreted from enteroendocrine L-cells orchestrate the fate of ingested nutrients. Glucagon-like peptide-1 (GLP-1) acts as an incretin, thus boosting postprandial insulin secretion, and both GLP-1 and peptide YY (PYY) inhibit further food intake. Using a transgenic mouse model fluorescently labelling proglucagon expressing cells, we identified insulin-like peptide-5 (InsI5) as another hormone expressed in colonic L-cells, and characterised its role in murine physiology.

Materials and methods: Expression of InsI5 was investigated by RT-PCR and immunohisto-chemistry. Plasma levels of InsI5 after fasting and re-feeding were assessed by ELISA (Kamiya). Wild-type and newly generated InsI5-receptor (relaxin/insulin-like family peptide receptor-4) knock-out (Rxfp4^{-/-}) mice, accustomed to fasting and/or i.p. injections, were injected with InsI5 (either from Phoenix or chemically synthesised in Melbourne) or polyclonal InsI5 antibodies (Phoenix) and assessed for food intake. All animal studies were in accordance with the UK Home Office legislation and approved by the appropriate ethical committee.

Results: RT-PCR detected specific InsI5 expression in colonic, but not duodenal/jejunal L-cells and immunofluorescence microscopy confirmed co-expression with proglucagon in an overlapping set of vesicles. In spite of a previous report of reduced insulin responses in InsI5^{-/-} mice, we failed to detect significant effects of InsI5 on insulin secretion from isolated islets, and Rxfp4^{-/-} mice did not exhibit impaired glucose tolerance. However, we observed that over-night fasted mice had elevated plasma InsI5 levels (4.2 \pm 0.5 pg/ml) which fell after 10 hrs of re-feeding (2.7 \pm 0.2 pg/ml, n = 10). This effect was pronounced in mice restricted to 60% caloric intake for 2 weeks (32.7 \pm 6.4 pg/ml fasting; 2.5 \pm 0.2 pg/ml 10 h refed, n=10) but blunted in mice receiving a 45% high-fat diet, consistent with the finding that colonic InsI5 mRNA expression was elevated in the calorie restricted group. Over-night fasted mice injected with InsI5 (0, 8, 40, 200 or 1000 ng/25g body weight) showed a dose dependent increase in food intake (225 \pm 25 mg chow/20 min at the highest dose compared to 13 \pm 12.5 mg chow/20 min in the placebo group). This effect was absent in Rxfp4^{-/-} mice. Tail vein injection with polyclonal InsI5 antibodies blunted re-feeding responses in fasted mice (5.8 \pm 0.2 g cumulative food intake/24 h at 100 μ g InsI5-IgG/mouse compared to 7.1 \pm 0.1 g after injection of an unspecific control IgG, n= 5 respectively) and this effect was also absent in Rxfp4^{-/-} mice.

Conclusion: These results are consistent with InsI5 acting as an orexigenic peptide secreted from the distal intestine, joining the only other established orexigenic gut-peptide, ghrelin, which is secreted from the stomach. Co-expression in a subset of anorexigenic peptide (GLP-1 and PYY) secreting L-cells is surprising and requires further investigations.

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Mechanism and physiological roles of glucose sensing in enteroendocrine cells

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Background and aims: Glucagon-like peptide 1 (GLP-1) is secreted from enteroendocrine L-cells in response to oral glucose ingestion and plays an important role in dampening the rise in postprandial blood glucose levels. However, the mechanism of glucose sensing in the L-cells and its physiological consequence is not fully understood. In this study, the mechanism of glucose sensing in the L-cells and the physiological importance of luminal glucose sensing in the maintenance of glucose homeostasis was examined in mice *in vivo*.

Materials and methods: C57BL/6 mice were administrated with carbohydrates (glucose or maltose) together with or without an antagonist of SGLT1 (phloridzin) or GLUT2 (phloretin) and the portal blood was collected and subjected to GLP-1 assay. The activation of enteroendocrine cells and neurons in the brainstem was detected by immunostaining of phospho-CaMK2. In addition, glucose-induced suppression of gluconeogenesis was evaluated by both oral (p.o.) and intraperitoneal (i.p.) administration.

Results: Significant GLP-1 secretion was induced by intraduodenal glucose administration at 5 min after infusion. The secretion was significantly blocked by co-administration of phloridzin but not by phloretin. I.p. glucose administration failed to trigger GLP-1 secretion. While plasma GLP-1 concentration was not increased at 30 min after oral glucose administration, it was significantly increased by oral maltose. Interestingly, GLP-1 secretion at 30 min after maltose administration was not suppressed by phloridzin, suggesting that glucose-sensing mechanism in L-cells differs between early and late phases after stimulation. Immunostaining of duodenum revealed that enteroendocrine cells and small numbers of nonenterothelial cells in the villi were detected positive for phospho-CaMK2 at 5 min after intraduodenal glucose administration. Similarly, several cells in the medulla oblongata were shown to be activated by intraduodenal glucose administration. We also examined the gluconeogenesis and STAT3 phosphorylation in the liver in response to either p.o. or i.p. glucose administration. Interestingly, the suppression of gluconeogenesis and induction of STAT3 phosphorylation by p.o. administration was significantly potent than that by i.p. administration.

Conclusion: Glucose sensing in L-cells is dependent on SGLT1-mediated signaling in the early phase but becomes independent in the late phase after luminal glucose stimulation. Our data suggested that glucose in the gut lumen first activates enteroendocrine cells and then the information is transferred to several nuclei in the brain stem, influencing the glucose metabolism in the liver.

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Free fatty acid receptor GPR120 is highly expressed in enteroendocrine K cells of upper small intestine and has a critical role in GIP secretion after fat ingestion

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Background and aims: Gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K-cells in response to fat and glucose ingestion. Recently, free fatty acid receptor (FFAR) GPR120 was identified as a “lipid sensor” and was involved in GLP-1 secretion. However, GPR120 expression and role in K-cells remain unclear. In this study, we elucidated GPR120 gene expression in K-cells using GIP-GFP knock-in (GIP-GFP) mice in which K-cells can be visualized by GFP fluorescence. Furthermore, we clarified the effects of GPR120 on GIP secretion using GPR120-deficient mice (GPR120^{-/-}) and GPR120 antagonist.

Materials and methods: The number of GFP-positive cells was measured by flow cytometry analysis and immunohistochemistry. GFP-positive cells and GFP-negative cells were collected as K-cells and non-K-cells, respectively, from GI tract of GIP-GFP mice by flow cytometer. GIP content in GFP-positive and GFP-negative cells were measured by ELISA. GIP mRNA and FFAR mRNA expressions (GPR40, GPR41, GPR43, GPR119, and GPR120) in GFP-positive and GFP-negative cells were assessed by sq RT-PCR. Oral glucose tolerance tests (OGTTs) and lard tolerance tests (OLTs) were performed using GPR120^{-/-} mice and wild-type (WT) mice to evaluate total GIP secretion. After oral or intravenous administration of GPR120 antagonist in C57/BL6 mice, total GIP levels during OLTs were measured.

Results: GFP-positive cells were observed in small intestine, but not in stomach and colon. K-cell number and GIP content and mRNA expression in K-cells were significantly higher in upper small intestine than those in lower small intestine. GPR120 mRNA was highly expressed in K-cells (but not in GFP-negative cells) of upper small intestine, while GPR40 mRNA and GPR41 mRNA were highly expressed in K-cells (but not in GFP-negative cells) of lower small intestine. GPR120^{-/-} mice had lower GIP secretion (75% reduction) during OLT compared to WT mice, but not during OGTT. Oral administration of GPR120 antagonist significantly attenuated lard oil-induced GIP secretion. On the other hand, intravenous administration of GPR120 antagonist did not reduce GIP levels after lard oil ingestion.

Conclusion: We confirmed that GPR120 is substantially expressed in K cells of the upper small intestine. Our results using GPR120^{-/-} mice and a GPR120 antagonist demonstrate that GPR120 plays a critical role in lipid-induced GIP secretion from K-cells population located mainly in upper small intestine.

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CART is a regulator of GIP and GLP-1 expression and secretion in vitro

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a regulatory peptide that controls islet hormone secretion and beta-cell survival. We have recently shown that CART is expressed in human enteroendocrine cells, including L- and K-cells in the human duodenum and jejunum. Furthermore, we have shown that CART plasma levels increase after a meal in humans. In the present study we aimed to examine whether endogenous L- and K-cell CART regulates incretin hormone secretion and expression.

Materials and methods: CART gene expression was silenced using siRNA in GLUTag and STC-1 cells, used as L- and K-cell models respectively. Gene expression was assessed with qPCR, protein content and secretion with ELISA, and cell survival using WST-1 assay.

Results: CART silencing resulted in a 84.6±5.9% reduction in CART mRNA levels in GLUTag cells (p<0.001) and in a 58.5±8.1% reduction of CART mRNA in STC-1 cells (p<0.001). This was paralleled by increased incretin gene expression in both GLUTag and STC-1 cells. In GLUTag cells GLP-1 mRNA expression was increased by 133.7±12.5% after CART silencing (p<0.001). This corresponded to 2.3±0.8 fold increase in GLP-1 (active) content (p<0.05). In STC-1 cells silencing of CART increased mRNA levels of both GLP-1 and GIP by 147±7.1% and 125±8% respectively (p<0.001). In

addition, silencing of CART provoked increased GLP-1 (active) secretion in GLUTag cells stimulated with 2.8 mM glucose, 16.7 mM glucose and IBMX and 16.7 mM glucose (3.79±0.15, 3.78±0.32 and 4.45±0.32-fold respectively, p<0.001). Moreover, CART silencing provoked 1.71±0.39-fold (P<0.05) increased cell survival in GLUTag cells.

Conclusion: We conclude that CART expressed in L-cells and K-cells acts as a regulator of incretin synthesis and secretion.

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In vivo beta cell glucose sensitivity regulates ex vivo islet size, transdifferentiation and GLP1 immunoreactivity in the alpha cells in humans

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Background and aims: Neogenesis from duct cells, increased islet size and trans-differentiation of α cells to β cells likely represent compensatory mechanisms to cope with the increased insulin demand in insulin resistance states. Following pancreateoduodenectomy healthy patients displays an increase in GLP-1 secretion, the mechanism contributing to greater GLP-1 secretion is not fully understood and suggests either hypersecretion by existing intestinal L-cells and/or other potential sources of the incretin hormone. Among those, α cells are a potential source of the incretin hormone.

Materials and methods: To investigate the relation between in-vivo insulin secretion (IS), sensitivity and incretin effect with ex-vivo islet characteristics, 18 non-diabetic patients (10 F/8 M, 51±15 yrs., BMI 27.9±5.3 kg/m²) scheduled for pancreateoduodenectomy underwent a 2-h hyperglycemic clamp, a mixed meal test and an hyperinsulinemic euglycemic clamp. β cell glucose sensitivity (GS) was calculated as the ratio of insulin secretion and glucose increments, both during the hyperglycemic clamp and mixed meal test. The incretin effect was estimated as the ratio of glucose sensitivity during the HC and MMT. Pancreas samples were collected during surgery for IHC for glucagon, insulin and GLP1+ cells to assess islet morphology. Neogenesis was evaluated by the quantification of scattered islets and ductal cells by CK19 immunostaining and trans-differentiation of α to β cell was quantified by counting the % of insulin and glucagon double positive cells.

Results: When subjects were classified as insulin sensitive or resistant (using the median glucose uptake during the euglycemic clamp [4.9 mg•kg⁻¹•min⁻²] as a cut-off), insulin resistant subjects displayed a significantly higher basal IS (IR 127±23.7 vs. IS 91.7±6.78 pmol•min⁻¹•m⁻², p=0.04), whereas GS during HC (IR 47.7±10.5 vs. IS 91.5±17.4 pmol•min⁻¹•m⁻²•mM⁻¹, p=0.03) was significantly lower. Analysis of the entire group revealed an inverse correlation between islet size and GS (r= -0.49; p<0.05) and GU, and between GS and % of double+ cells (r= -0.72; p=0.03). No correlation was found between neogenesis markers and GS. Following the pancreateoduodenectomy, the incretin effect was significantly decreased in the insulin sensitive patients (Incretin effect before surgery 3.21±0.88 vs. after surgery 1.48±0.31, p=0.05), whereas no changes were observed in the insulinresistant subjects (Incretin effect before surgery 2.03±0.41 vs. after surgery 2.16±0.96, p=NS).

Conclusion: Our data suggest that insulin resistance determines alterations in islet morphology and β cell function, with a strict correlation between function and morphology. Impaired β cell GS might represent a major stimulus for the induction of trans-differentiation of α to β cells and increased islet size. Further, relative compensation of incretin effect following PD may be driven by the increased α mass and the GLP1 immunoreactivity in the α cells observed in insulinresistance.

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Effect of acute GIP- and GLP-1- infusion on circulating levels of pro-atrial natriuretic peptide in subjects with different stages of glucose tolerance

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Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonist acts antihypertensive via release of atrial natriuretic peptide in mice. Moreover, reduction of glycemic load by alpha-glucosidase inhibitors increase circulating levels of pro-atrial natriuretic peptide (proANP) suggesting an evidence of gut-heart axis also in humans. Whether GLP-1 and glucose-dependent insulinotropic peptide (GIP) interact with NP system in humans is unknown.

Materials and methods: Twelve patients with type 2 diabetes (T2DM) (nine men and three women; 61 ± 10 years; BMI 30.0 ± 3.7 kg/m²; HbA(1c) 7.3 ± 1.5%) were studied. In randomized order, intravenous infusions of GLP-1(7-36)-amide (1.2 pmol • kg⁻¹ • min⁻¹), GIP (4 pmol • kg⁻¹ • min⁻¹), GLP-1 plus GIP, and placebo were administered over 360 min after an overnight fast (≥ 1 day wash-out period between experiments). Additionally, effect of 240 min GIP-infusion (2 pmol • kg⁻¹ • min⁻¹) on proANP was studied in glucose-tolerant male subjects (n=8). Plasma proANP concentrations were measured by an automated mid-region-directed proANP immunoassay.

Results: GLP-1 decreased proANP levels (mean ± sem, 44.1 ± 3.1 vs. 34.2 ± 3.0 pmol/l; p < 0.05). During placebo, GIP and GIP + GLP-1 infusion no significant changes of circulating proANP were observed in T2DM subjects. In glucose tolerant subjects, proANP levels are decreased at the end of the experiments with placebo and GIP (51.9 ± 6.5 vs. 40.0 ± 5.4 pmol/l in placebo, p < 0.05; 49.5 ± 6.3 vs. 40.8 ± 6.5 pmol/l in GIP-tests, p = 0.051).

Conclusion: As proANP concentration reflects ANP secretion, our data could not confirm interactions between two major incretins and ANP in humans.

Clinical Trial Registration Number: NCT00774488

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Novel GIP receptor-mediated bioassay more accurately reflects changes of GIP activity than traditional assays

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Background and aims: GIP and GLP-1 are incretins released from the gut rapidly inactivated by dipeptidyl peptidase-4 (DPP-4) after secretion into truncated forms that are no longer insulinotropic forms of incretins. Although recently developed ELISA kits may be able to measure active incretins levels, it is unclear if the available assays detect totally biological active forms. Moreover, recent reports suggest GIP has a short-form isoform GIP (1-30) secreted from gut and pancreas, which may not be detectable by the available methods. Therefore, we determined active GIP and GLP-1 levels from human blood samples using novel cell-based, receptor-mediated bioassays.

Materials and methods: We utilized the cell lines stably co-transfected with human-form GIP or GLP-1 receptors and a cAMP-inducible luciferase expression construct for bioassays. We performed a 75 g OGTT in six non-diabetic subjects and measured plasma total GIP (Millipore), total GLP-1 (Millipore), insulin, C-peptide and glucagon levels by ELISA, active GIP and active GLP-1 levels by bioassays. Next, we performed another OGTT in identical subjects after administration of DPP-4 inhibitor (sitagliptin: 100 mg/day) for three days. We measured plasma total/active GIP and GLP-1 levels as above. Additionally, we compared the efficacy of our GIP bioassay with that of a commercial active GIP ELISA kit (IBL, Japan) by measuring samples from identical subjects before and after sitagliptin administration.

Results: To evaluate the specificity of the bioassays, we confirmed their responsiveness with several synthetic incretin receptor agonists. In the GIP bioassay, GIP (1-42) and short-form GIP (1-30) almost equivalently increased luciferase activity in a concentration dependent manner. In the GLP-1 bioassay, exendin-4 produced almost comparable luciferase activity to GLP-1

(7-36 amide) but liraglutide did less than GLP-1 (7-36 amide). In samples collected during on OGTT, total GIP levels rapidly increased from 0 min to 15 min and gradually increased up to 120 min (68.8 ± 11.4 pmol/l). In contrast, active GIP levels peaked at 30 min (42.3 ± 4.3 pmol/l) and dropped at 60 min. Notably, active GIP had a positive correlation with the insulin levels. Total GLP-1 levels increased to 30 min and remained at similar levels up to 120 min (25.4 ± 2.7 pmol/l). In contrast, active GLP-1 levels reached a peak at 30 min and then dropped. Administration of a DPP-4 inhibitor to identical subjects induced 1.4-fold increase of active GLP-1 levels at 15 minutes after oral glucose load. Interestingly, active GIP measured by bioassay levels increased approximately by 20-fold after DPP-4 treatment. But total and active GIP levels by ELISA were increased just 1.7 and 2.1-fold, respectively. Other DPP-4 inhibitors (linagliptin: 5 mg/day and vildagliptin: 100 mg/day) showed similar effect to sitagliptin. Then we investigated the discrepancy in measured active GIP levels between bioassay and ELISA. We first confirmed that Sitagliptin per se did not increase luciferase activity. The bioassay could detect short-form GIP (1-30), but total or active ELISA kits could not at all.

Conclusion: We propose that the bioassays more accurately reflect the contribution of the incretins in the entero-insular axis and short-form GIP (1-30) may be more important than GIP (1-42) in DPP-4 inhibitor treatment.

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PS 030 Gut endocrinology in vivo

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Effects of a non-glucose food preload on oral glucose tolerance in subjects with impaired glucose tolerance and type 2 diabetes

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Background and aims: Protein and fat ingestion delays gastric emptying and stimulates gut hormones release. Recent studies have suggested that these effects could be exploited to improve the plasma glucose excursions induced by carbohydrates in type 2 diabetic patients (T2D). The present study was designed to measure the impact of a small non-glucose food preload on the major components of glucose homeostasis in subjects with impaired glucose tolerance (IGT) and T2D and to test whether gut hormones participate in this response.

Materials and methods: Ten diet-controlled T2D patients (8 males, age 55±7 years, BMI 28.7±4.7 kg/m², HbA1c 48±2 mmol/l) and thirteen IGT subjects (7 males, age 47±5 years, BMI 26.3±1.6 kg/m², HbA1c 38±1 mmol/l) were enrolled. On two separate days, after an overnight fast, they were randomised to a preload of either 500 ml of water (control) or Parmesan cheese (50 g) plus a boiled egg with 300 ml of water; 30 minutes later they underwent a standard 75 gr oral glucose tolerance test (OGTT). Arterialised timed blood samples were collected to measure plasma glucose, insulin, C-peptide, GLP-1 and GIP. Two stable glucose tracers were administered ([6,6-²H₂]glucose and [U-¹³C]glucose, *i.v.* and *per os* respectively) to measure the rate of appearance of ingested (RaO) and endogenous glucose and the glucose clearance. Three major components of β -cell function, namely glucose sensitivity (β GS), rate sensitivity (β RS) and potentiation (POT) were evaluated by modeling insulin secretion (estimated from C-peptide deconvolution) and plasma glucose. Insulin sensitivity was estimated using the OGIS method.

Results: In T2D patients, the plasma glucose rise during the OGTT was significantly lower after the food preload than after water ingestion (iAUC -49.4±5.9%, $p<0.0002$). The food preload produced a delay in glucose absorption (RaO iAUC -27±12%, $p<0.05$), an enhancement of β GS (+102±31%, $p<0.006$), and an increase in GLP-1 (iAUC +176±49%, $p<0.004$) and GIP response (iAUC +74±31%, $p<0.03$), while insulin sensitivity, glucose clearance, endogenous glucose production, β RS and POT were not affected. In IGT subjects, the effect of the food preload on glucose tolerance was less pronounced (iAUC -39±7%, $p<0.0002$) and explained by a significant improvement in POT (+43±17%, $p<0.04$) and a minor delay in glucose absorption (RaO iAUC -16±8%, $p<0.01$). While the GLP-1 response was similarly enhanced (iAUC +149±58%, $p<0.002$), GIP secretion in IGTs was stimulated to a greater extent (iAUC +179±49%, $p<0.001$). In the whole dataset, glucose tolerance improved in proportion to its baseline derangement and was not correlated to the changes in either GLP-1 or GIP.

Conclusion: A small non-glucose food preload significantly improves glucose tolerance in diet-controlled T2D and IGT. This results from different combinations of delayed glucose absorption and improved β -cell function in the two study groups. Simple diet recommendations have the potential to control postprandial hyperglycaemia in T2D patients by engaging mechanisms, other than gut hormones, that appear to be preserved in the early stages of the disease.

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Relationship between meal induced glucagon-like peptide-1 response and metabolic syndrome prevalence in type 1 diabetic patients

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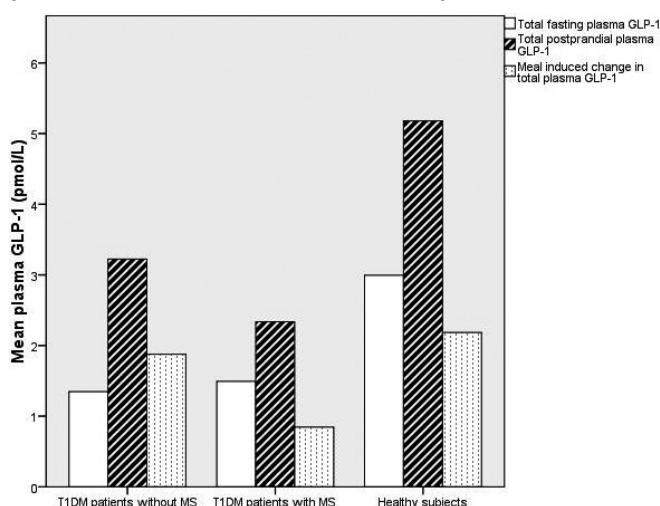
Background and aims: Metabolic syndrome (MS) can be found in about 30–40 % of type 1 diabetic (T1DM). While there is a growing body of evidence suggesting that meal induced glucagon-like peptide-1 (GLP-1) response in type 2 diabetic (T2DM) patients is diminished, GLP-1 secretion in T1DM and population with MS was not extensively studied. We aimed to analyse the

relationship between fasting total GLP-1 level, meal induced GLP-1 response and MS prevalence in T1DM patients.

Materials and methods: A total of 77 T1DM patients (61% male, mean age 46 years, median BMI 25 kg/m², median diabetes duration of 21 years) were included. MS was diagnosed in 26 (33.76%) of them according to IDF (International Diabetes Federation) definition (2009 year) including at least two of the following criteria: waist circumference >80 cm in female (94 in male), triglycerides above 1.7 mmol/L or specific treatment, HDL cholesterol <1.03 mmol/L in males and 130 or diastolic >85 mm Hg, or specific treatment, fasting plasma glucose (FPG): > 5.6 mmol/L, or previously diagnosed T2DM. The control group included 10 normal glucose tolerance (NGT) subjects adjusted for age, gender and BMI. Circulating GLP-1 level was measured before and 30 min after a test meal. The test meal (324 (324–528) kcal) comprised about 20% fat, 60% carbohydrate, and 20% protein. Total GLP-1 level was measured by ELISA using ALPCO Diagnostics kit. The change between 30 minutes postprandial and fasting GLP-1 concentration was calculated: Δ GLP-1=postprandial GLP-1-fasting GLP-1 concentration.

Results: T1DM patients showed significantly lower total fasting, postprandial and meal induced GLP-1 level compared to NGT group. Between T1DM patients significantly higher rise in circulating GLP-1 level was noticed in group without compared to those with MS (1.89±2.25 pmol/L vs 0.77±1.74 pmol/L, $p=0.017$). Between the T1DM without MS and NGT group a significant difference in total fasting GLP-1 (1.34±1.39, 2.99±1.34, $p=0.003$), postprandial GLP-1 level (3.24±2.43, 5.18±2.85, $p=0.017$) and Δ GLP-1 (1.89±2.25, 2.17±1.66, $p=0.032$) was found. The difference in meal induced change between these two groups was not significant ($p=0.487$). In the multivariate logistic regression analysis, Δ GLP-1 concentration was significantly inversely associated with the presence of MS after adjustment for age, sex, duration of diabetes and calorie intake (0.704 (0.528–0.956)).

Conclusion: In T1DM patients higher meal induced GLP-1 response is inversely related to the presence of MS. The possible role of GLP-1 in the pathogenesis of MS in T1DM needs to be further investigated.



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Intravenous arginine stimulates GLP-1 release across spectrum of glucose tolerance

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Background and aims: Meal-induced secretion of GLP-1 has been well-described, yet limited data exist on *i.v.* secretagogues of GLP-1. As part of a series of experiments to characterize beta cell response to *i.v.* arginine (arg), we previously showed that insulin secretion (AIRarg) responses to arg differed

across glucose tolerance (GT) groups. In the same subjects we report total GLP-1 responses to i.v. arg in 53 obese subjects with normal glucose tolerance (NGT), prediabetes (PDM), and type 2 diabetes (T2DM).

Materials and methods: Following an overnight fast, samples were acquire for total GLP-1 pre- and for 10 min post an i.v. arg bolus (5 gm over 30 sec) at basal glucose. Pre-and post-arg GLP-1 samples were also acquired during the last 10 min of a 60 min glucose infusion (900 mg/min).

Results: The table summarizes results during basal glucose period; previously reported and baseline adjusted AIRarg insulin responses are also included. Pre-arg fasting GLP-1 differed across GT spectrum, highest in T2DM. ARG elicited GLP-1 secretion in all 3 GTs, with greater changes in PDM and T2DM than NGT (Delta GLP-1). Pre-arg GLP-1 did not correlate with AIRarg, whereas change in GLP-1 correlated with AIRarg for NGT and T2DM but not PDM in basal glucose state (NGT/T2DM: $r = 0.52/0.49$ $P < 0.02$; PDM $r = 0.16$ NS). Similar responses observed during high glucose infusion (not shown).

Conclusion: We conclude that 1) i.v. arginine acutely stimulates GLP-1 secretion irrespective of glucose tolerance status; 2) Pre-arginine GLP-1 was not associated with insulin secretory response, but GLP-1 secretion after arginine is positively associated with insulin secretion in NGT and T2DM.

	N	BMI (mean±SD)	Basal Glucose (mMol) (mean±SD)	AIRarg (pM) Geometric mean (95% CI)	GLP-1 pre- ARG (pM) Geometric mean (95% CI)	GLP-1 post-ARG (pM) Geometric mean (95% CI)	Delta GLP-1 (pM) Mean (95% CI)
NGT	23 (12M/11W)	31.5±2.8	5.2±0.3	582 (488, 694)	3.8 (3.2, 4.6)	4.9 (4.3, 5.7)	1.1 (0.6, 1.5)
PDM	8 (2M/6W)	33.0±2.6	6.2±0.3	638 (444, 916)	3.8 (2.3, 6.3)	6.7 (5.2, 8.5)	2.6 *** (1.8, 3.4)
T2DM	22 (11M/11W)	32.8±3.9	8.7±0.3#	244# (191, 311)	7.4 *** (6.0, 9.0)	9.1 *** (7.5, 11.0)	1.8* (1.3, 2.3)
ANOVA across populations (<i>P</i>)			<0.001	<0.001	<0.001	<0.001	<0.01
* = $P < 0.05$ v NGT							
** $P < 0.01$ v NGT							
*** $P < 0.001$ v NGT							
# $p < 0.001$ vs NGT and PDM							

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Effects of region of small intestine exposed to glucose on incretin hormones, insulinaemia and glycaemia in healthy older subjects

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Background and aims: The release of incretin hormones is pivotal to post-prandial glucose homeostasis. We have reported that the stimulation of glucagon-like peptide-1 (GLP-1), but not glucose-dependent insulinotropic peptide (GIP), is dependent on the length of small intestine exposed to glucose, but the impact of the region of small intestine remains uncertain. The aims of this study were to determine the incretin, insulinaemic and glycaemic responses to intraduodenal (ID) glucose when the proximal small intestinal region (~60cm from the pylorus) is bypassed.

Materials and methods: Ten healthy older subjects (9M,1F; age 65-79yr) were studied on 2 separate occasions after an overnight fast, in random order. On each occasion they were intubated with a custom-designed catheter incorporating an intraduodenal (ID) balloon to isolate proximal (<60cm from pylorus) small intestinal regions. Each subject then received ID glucose (3kcal/min) into either a) both the proximal and distal regions "GPD", or b) the distal region only "GD" for 60min. On the GPD study day, glucose was infused into the proximal region and aspirated every 10min, so that the amount of residual glucose to be infused into the distal region could be calculated. On the GD study day, 0.9% saline was infused concurrently into the proximal region. Blood glucose and plasma insulin, total GIP and GLP-1 were measured between t=0-60min, and the insulin/glucose ratio at 60min calculated. Data were analysed using Student's paired t-tests and expressed as mean ± SEM.

Results: There were rises in blood glucose and plasma insulin, GIP and GLP-1 on both days ($P < 0.01$). When the responses to GD and GPD were compared there was no significant difference in blood glucose, or plasma in-

sulin, although mean values for blood glucose were less, and plasma insulin greater, with GD and the insulin/glucose ratio at 60min was greater with GD (11.8 ± 2.3 vs 7.8 ± 1.2 , $P < 0.05$). The stimulation of GIP was less ($P < 0.01$), and that of GLP-1 greater ($P < 0.05$), with GD.

Conclusion: We conclude that the effects of enteral glucose on GIP and GLP-1 are dependent on the region of small intestine exposed with a consequent impact on insulinaemia.

Table: Blood glucose and plasma insulin, GIP and GLP-1

	60min		Peak		AUC 0 - 60 min	
	GD	GPD	GD	GPD	GD	GPD
Blood glucose, mmol/L	9.5±0.6	10.3±0.5	9.6±0.6	10.3±0.5	436±134	449±13
Plasma insulin, mU/L	44.4±7.2	37.3±4.5	44.4±7.2	37.3±4.5	1143±149	900±132
Plasma GIP, pmol/L	55.0±5.2*	68.8±4.9	55.8±5.1*	70.1±4.6	2669±241	2893±212
Plasma GLP-1, pmol/L	68.6±6.8*	57.1±7.0	75.7±8.5*	58.5±6.9	2934±362*	2110±201

Values are mean ± SE. GD = saline proximal/glucose distal; GPD = glucose proximal and distal * $P < 0.05$, GD vs GPD.

Supported by: NHMRC

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Encapsulated glutamine significantly increases circulating concentrations of glucagon-like peptide-1 and insulin and increases meal size in healthy volunteers

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Background and aims: Type 2 diabetes (T2D) is now a major cause of death and disability worldwide. At present, bariatric surgery is the only treatment capable of inducing diabetes remission, possibly due to enhanced nutrient delivery to the distal gut, a phenomenon which is associated with marked increases in the secretion of gut hormones, such as glucagon-like peptide 1 (GLP-1). However, the use of bariatric surgery is limited by expense and invasiveness and there is an urgent need to identify safe and affordable non-surgical treatments which can promote similar effects. Previous work in vitro has identified that glutamine is a potent secretagogue of GLP-1. The aim of the current study was to identify if encapsulated glutamine can increase endogenous secretion of GLP-1 in human volunteers using a delivery system which targets glutamine release to the ileum. A secondary aim was to assess the effect of the encapsulated glutamine on glucose tolerance and meal size.

Materials and methods: A randomized, double blind, placebo-controlled cross-over study was performed to assess the effects of a single dose of encapsulated glutamine (6g, 3.6g or placebo) on venous concentrations of total GLP-1 in fasting healthy volunteers. Glutamine was encapsulated by a company specialising in drug delivery. Each capsule was coated with Acryl-Eze to promote capsule release around the mid ileum. Capsules were made using GMP-grade materials and were tested for stability to determine the shelf life. The effects of capsule ingestion upon glucose tolerance and meal size were studied using a standard 75g oral glucose tolerance test and ad libitum meal respectively. The study received ethical approval from the local research ethics committee and all participants gave written informed consent. Statistical analysis was performed using the paired t test.

Results: 29 participants were recruited in total (14 male, 15 female; age 22-58 years, body mass index $18.5 - 31.8$ kg/m²). Ingestion of 6g glutamine was associated with increased concentrations of GLP-1 after 90 minutes ($n=10$; 3.6 pmol/l vs 2.2 pmol/l for placebo; $p=0.006$), increased concentrations of insulin after 90 minutes ($n=10$; 62.0 pmol/l vs 50.9 pmol/l; $p=0.047$) and increased meal size at 120 minutes ($n=10$; 542 g vs 481 g eaten; $p=0.008$). No safety concerns were identified following the ingestion of encapsulated glutamine.

Conclusion: A single oral dose of encapsulated glutamine is able to promote increased secretion of GLP-1 and is associated with increased insulin release. However, this promoted increased meal size, an undesirable effect, perhaps because the orexigenic effects of insulin release predominated over the anorexigenic effects of GLP-1 release following administration of glutamine. In conclusion, GLP-1 release was promoted in vivo using a single dose of encapsulated glutamine but the effect size was small and was not associated with beneficial metabolic effects.

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Gallbladder emptying and single-dose metformin elicit robust and additive glucagon-like peptide-1 responses

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Background and aims: Preclinical studies suggest that gallbladder emptying and subsequent activation of the bile acid receptor TGR5 on enteroendocrine L cells leads to glucagon-like peptide-1 (GLP-1) secretion. Drugs affecting bile acid binding (colesevelam (COL)) or reabsorption (metformin (MET)) seem to increase postprandial GLP-1 secretion in humans. We hypothesised that gallbladder emptying stimulates human GLP-1 secretion and that COL and MET, respectively, would potentiate any GLP-1 secretion induced by gallbladder emptying.

Materials and methods: Ten subjects (age (mean±SD): 23.4±3.8 years; BMI: 21.9±1.8 kg/m²; HbA1c: 5.1±0.3%) were studied on 6 randomised days. In a double-blind fashion the subjects received 1) COL (3.75 g); 2) MET (1.5 g); or 3) placebo (PLA) in 50 ml water admixed 1.5 g paracetamol (for evaluation of gastric emptying) administered via nasogastric tube, with a concomitant 60-minute iv infusion of saline and cholecystokinin-8 (CCK), respectively. Blood was sampled for 4 hours for measurements of plasma GLP-1, glucose, insulin, C-peptide and glucagon. Gallbladder emptying was measured by ultrasound. Food intake was assessed at the end of each day.

Results: CCK infusion during PLA induced complete gallbladder emptying and a significant GLP-1 response (incremental AUC) compared to saline infusion (392±173 (mean±SEM) vs. -277±94 pM×min, p=0.02). MET without CCK elicited a significant GLP-1 response (215±87 vs. -277±94 pM×min (saline+PLA), p=0.002), which was potentiated by CCK-induced gallbladder emptying (963±202 pM×min (CCK+MET), p=0.03). COL did not elicit significant GLP-1 responses. Plasma glucose was not affected by the interventions, nor was insulin, C-peptide, glucagon or food intake.

Conclusion: CCK-induced gallbladder emptying and single dose metformin, respectively, elicit robust and additive GLP-1 responses in humans. We, therefore, speculate that metformins mode of action includes stimulation of GLP-1 secretion by both bile acid-dependent and independent mechanisms.

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Effect of the bile acid chenodeoxycholic acid and the bile acid sequestrant colesevelam on glucose metabolism in patients with type 2 diabetes and in healthy control subjects

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Background and aims: In patients with type 2 diabetes, rectal administration of bile acids, and oral bile acid sequestrants lower plasma glucose (PG). We evaluated the effects of chenodeoxycholic acid (CDCA) and the bile acid sequestrant colesevelam (COL), delivered by intragastric tube, on glucagon-like peptide-1 (GLP-1) (primary endpoint), PG, insulin, C-peptide, glucagon, cholecystokinin (CCK), gastric emptying, gallbladder volume, appetite and food intake, in a placebo-controlled, double-blinded study.

Materials and methods: On 4 separate days 10 patients with type 2 diabetes (age (mean±SD): 62±7 years, BMI: 28.9±2.3 kg/m²; HbA1c: 53±12 mmol/mol) and 10 matched healthy control subjects (age: 61±10 years, BMI: 28.2±3.2 kg/m²; HbA1c: 37±4 mmol/mol) received 1) CDCA (1.25 g); 2) COL (3.75 g); 3) CDCA+COL, or 4) placebo; suspended in 100 ml of water with 1.5 g paracetamol (for evaluation of gastric emptying). During 180 min blood was drawn, gallbladder volume was evaluated by ultrasound, and appetite perception was evaluated by visual analogue scale. At the end of each day ad libitum food intake was evaluated.

Results: In both the patients with type 2 diabetes and the healthy subjects, CDCA elicited a significant increase in GLP-1 vs. COL, CDCA+COL and placebo (p<0.05). While none of the interventions changed PG, CDCA elicited

a small but significant increase in C-peptide/PG ratio vs. COL, CDCA+COL and placebo in both groups. CDCA increased glucagon in both groups vs. COL, CDCA+COL and placebo. In the healthy control subjects, COL increased CCK significantly vs. CDCA, CDCA+COL and placebo, but not in patients with type 2 diabetes. CDCA slowed gastric emptying in both groups. COL tended to decrease gallbladder volume, whereas CDCA increased gallbladder volume. The interventions did not affect appetite perception or food intake.

Conclusion: Intragastric administration of CDCA increased GLP-1, insulin (measured as C-peptide/PG ratio) and glucagon secretion, and slowed gastric emptying. We speculate that the GLP-1 and subsequent insulin secretion is mediated by bile acid-induced TGR5 activation on L cells. Bile acid sequestration does not appear to have an acute effect on GLP-1 secretion in humans. *Clinical Trial Registration Number:* NCT01666223

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Early improvements in glucose metabolism after Roux-en-Y gastric bypass surgery are not explained by increased total bile acids or fibroblast growth factor 19

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Background and aims: Bile acids and fibroblast growth factor 19 (FGF19) have been suggested as key mediators of the dramatic improvements in glucose metabolism after Roux-en-Y gastric bypass (RYGB). The aim of this study is to describe fasting and postprandial total bile acid (TBA) and FGF19 concentrations before and after RYGB and relate them to parameters of glucose metabolism.

Materials and methods: In this prospective study, we enrolled 13 patients with type 2 diabetes (T2D) (BMI: 42.8 [95% c.i.: 40.1-45.7] kg/m²) and 12 subjects with normal glucose tolerance (NGT) (BMI: 41.3 [38.8-44.0] kg/m²). They were subjected to a 4-hour liquid meal test before, 1 week, 3 months and 1 year after RYGB. Blood was sampled for TBA and FGF19 as well as markers of glucose metabolism. Effects of time after surgery and group were analyzed by analysis of variance (ANOVA) in a linear mixed effects model using time from surgery and group (T2D or NGT) as fixed effects and subjects as random effect.

Results: Fasting TBA concentrations decreased 1 week after RYGB in NGT subjects but increased gradually thereafter in both groups with time from surgery (T2D: pre: 1.3 μmol/L [0.7-2.4], 1 week: 1.3 [0.7-2.3], 3 months: 1.5 [0.8-2.6], 1 year: 3.3 [2.3-4.7]; NGT: 1.6 [1.1-2.4], 0.8 [0.4-1.5], 2.3 [1.6-3.4], 2.6 [1.8-3.7], ANOVA ptime<0.001). Fasting FGF19 concentrations were unchanged after RYGB (ptime=0.6). Postprandial TBA concentrations were unchanged in T2D patients and decreased in NGT subjects 1 week after RYGB but increased afterwards with time from surgery (AUC TBA: T2D: 627 mmol x min/L [410-958], 481 [250-926], 1091 [829-1438], 1818 [1395-2368], NGT: 639 [521-784], 402 [320-505], 1338 [1051-1703], 1497 [1218-2368], ptime<0.001). Changes in AUC FGF19 followed the same pattern as AUC TBA (ptime<0.001). No significant group differences of fasting or postprandial TBA and FGF19 were found. HOMA-IR decreased, beta-cell function improved, and GLP-1 secretion greatly increased shortly (1 week) after RYGB.

Conclusion: The lack of change or even decline in TBA and FGF19 shortly after RYGB suggests that altered bile acid metabolism does not contribute to the acute improvements in glucose metabolism early after RYGB. Later changes may play a role on the longer term.

Clinical Trial Registration Number: NCT00810823

PS 031 Gut hormone action

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Inhibition of GIP receptor signalling in adipose tissue reduces insulin resistance in high fat diet-induced obesity

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Background and aims: Gastric inhibitory polypeptide (GIP) is an incretin that potentiates insulin secretion. GIP receptor (GIPR) is expressed not only in β -cells, but in adipose tissue as well, and previous in vitro studies show that GIP directly induces energy accumulation into the adipose tissue. Therefore, GIP has a direct and indirect effect (via insulin) on induction of adiposity. However, the effect of direct GIP action on adiposity in vivo remains unclear. In this study, we generated adipose tissue-specific GIPR-deficient mice (GIPRfat^{-/-}) and clarified the direct GIP action in adipose tissue in vivo.

Materials and methods: GIPRfat^{-/-} were generated from floxed GIPR mice (Lox) and adipocyte protein 2 (aP2)-Cre transgenic mice (aP2). GIPRfat^{-/-}, Lox, and aP2 were fed normal fat diet (NFD: 10% fat) or high fat diet (HFD: 60% fat) and their body weight gain was measured. After 15 weeks of NFD or HFD feeding, oral glucose tolerance test (OGTT), insulin tolerance test (ITT), and histological analyses (liver and adipose tissue) were performed. Fat volume (CT scan), energy expenditure, activity count, and triglyceride (TG) content in liver were measured.

Results: In GIPRfat^{-/-}, expression levels of GIPR mRNA were substantially lower in visceral and subcutaneous adipose tissue (90% reduction). Body weight gain, blood glucose levels, and insulin levels during OGTT did not differ among three types of mice under NFD condition. Under HFD condition, body weight gain was slightly lower in GIPRfat^{-/-} compared to that of control mice (Lox and aP2). Fat volume and adipocyte size did not differ between HFD-fed GIPRfat^{-/-} and control mice. OGTT data showed that insulin and glucose levels were significantly lower in HFD-fed GIPRfat^{-/-} compared to control mice. HOMA-IR and ITT data showed that insulin resistance was reduced in HFD-fed GIPRfat^{-/-}. Furthermore, fat content in liver was significantly lower in HFD-fed GIPRfat^{-/-} compared to control mice. Histological analysis showed that hepatic steatosis was reduced in HFD-fed GIPRfat^{-/-}. There was no significant difference in energy expenditure and mouse activity among three types of mice under HFD feeding.

Conclusion: GIP receptor signaling in the adipose tissue plays an important role in HFD-induced insulin resistance without increase in fat mass in vivo.

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The long-acting analogue of short-form GIP does not induce obesity in normal mice but ameliorates chronic hyperglycaemia in low-dose streptozotocin-induced diabetic mice

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Background and aims: It is still ambiguous whether GIP can be beneficial as potential therapeutics in the diabetic state. However, we reported that a short-isoform GIP, GIP (1-30), which is released chiefly from pancreatic alpha cells, may function not only as glucose-dependent insulin release in a paracrine manner, but also as the fetal islet development. Again, it is previously reported that GIP (1-30) less potentiates fat accumulation in contrast to GIP (1-42) secreted from the gut. To investigate the possibility of short-form GIP, we tested the efficacy of a long-acting GIP (1-30) analogue in vivo and investigated whether the analogue can suppress the progression to hyperglycemia and improve glycaemia in low-dose streptozotocin (LD-STZ) mice.

Materials and methods: We synthesized dipeptidyl peptidase-4 (DPP-4) resistant GIP (1-30) analogue via modification of the second position alanine and then attaching 40kDa polyethylene glycol (PEG) to the C-terminus. First, we administered PEGylated (350–7000 pmol/body) or non-PEGylated GIP to the non-diabetic control mice by the subcutaneous single injection, and evaluated pharmacokinetics of the peptides from 60 min until day 7. To measure plasma GIP activity, we employed the novel receptor-mediated bioassay reflecting cAMP production in vitro. Then, we used non-diabetic and LD-STZ-induced diabetic mice, with or without administration of the PEGylated GIP analogue. We designated mice to four groups, non-STZ,

STZ, non-STZ+GIP, STZ+GIP, respectively. We injected the PEGylated GIP analogue or vehicle subcutaneously every 3 days (initial dose 100 pmol/body and maintenance dose 70 pmol/body) and monitored body weight and non-fasted blood glucose until day 30. We measured HbA1c levels and conducted intraperitoneal glucose tolerance tests (IPGTT) at the end. Finally, we evaluated the islet morphology via double immunofluorescent staining with insulin and glucagon.

Results: The single subcutaneous injection of PEGylated GIP analogue gradually increased GIP activity in plasma but maintained much longer (the approximate half-life was 48 hours), whilst the injection of non-PEGylated analogue rapidly increased the activity but vanished within 2 hours. The maximum-dose injection of PEGylated GIP still augmented to detect biological GIP activity until day 7. Persistent PEGylated GIP treatment neither induced obesity nor affected glycemic levels in non-STZ groups. In contrast, PEGylated GIP treatment significantly decreased non-fasted blood glucose levels, reduced HbA1c levels (STZ+GIP: $4.9 \pm 0.2\%$ vs STZ: $5.7 \pm 0.4\%$, $p < 0.05$) and improved glucose excursion after IPGTT (STZ+GIP: 2465 ± 114 vs STZ: 2961 ± 166 min \times mmol/l, AUC glucose, $p < 0.01$) in LD-STZ mice. GIP treatment did not significantly increase insulin levels after IPGTT. However, GIP treatment ameliorated alpha cell expansion in the islets concomitant with suppressing elevated plasma glucagon levels observed in non-treated LD-STZ mice (STZ).

Conclusion: Our results indicate that the PEGylated short-form GIP analogue is possibly durable enough to suppress the progression to overt hyperglycemia with improving the islet function in LD-STZ-induced diabetic mice. Supported by: JSPS JDF

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Neural mechanisms contribute to islet effects of both GLP-1 and GIP

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Background and aims: After meal intake, the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) mediate 50–70% of β -cell insulin secretion. The rapid inactivation of the incretin hormones makes them very short-lived in the circulation which has questioned their role as circulatory hormones. Previous studies showing expression of GLP-1 receptors in vagal nerve cells and induction of electrical impulses in both afferent and efferent vagal nerve fibers by GLP-1 have instead indicated that the incretin hormones work through vagal nerve fibers. Therefore, the aim of this project was to examine whether blocking muscarinic receptors, i.e. the effector system of the cholinergic islet system, disrupts the insulinotropic effects of GLP-1 and GIP.

Materials and methods: Fasted anesthetized C57BL/6J female mice were given either saline or atropine methyl bromide (5 mg/kg) intraperitoneally 12 minutes before intravenous glucose administration (0.35 g/kg), co-injected with GLP-1 or GIP (both at 3 nmol/kg) via the tail vein. Blood was collected from the retrobulbar, intraorbital capillary plexus before and at 1, 5, 10, 20, 30 and 50 minutes after intravenous challenge for analysis of plasma glucose (glucose oxidase) and insulin (ELISA). Pancreatic islets were isolated by collagenase digestion and incubated (1h) in 2.8 and 11.1 mM glucose, with or without the presence of atropine (100 μ M), GLP-1 (100 nM), GIP (100 nM), atropine (100 μ M)/GLP-1 (100 nM) or atropine (100 μ M)/GIP (100 nM). The supernatant was analyzed for insulin.

Results: Atropine significantly reduced the insulin response to glucose + GLP-1 compared to saline (5 min: 1.2 ± 0.2 vs. 2.5 ± 0.4 nM, $P < 0.01$). Similarly, atropine also reduced the insulin response to glucose + GIP (5 min: 1.6 ± 0.3 vs. 2.7 ± 0.5 nM, $P < 0.05$ and 10 min: 0.6 ± 0.1 vs. 1.4 ± 0.3 nM, $P < 0.05$). Atropine did not affect the insulin response to glucose alone. In contrast, during islet incubations atropine did not affect the insulin secretory responses to GLP-1 or GIP.

Conclusion: We show here that blocking of muscarinic signaling by atropine markedly suppresses the insulinotropic effect of both GLP-1 and GIP in vivo but not in isolated islets. Based on these results, together with the finding of incretin hormone receptors on vagal nerve fibers, we therefore suggest that neural mechanisms contribute to the islet effects of both GLP-1 and GIP. Since only low levels of circulating intact incretin hormones reach the islets, parasympathetic vagal regulation of insulin secretion may therefore be an important component of the incretin effect.

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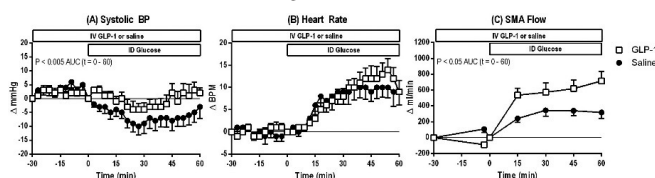
Effects of exogenous glucagon-like peptide-1 on the blood pressure, heart rate, mesenteric blood flow and glycaemic responses to intraduodenal glucose in older subjectsL.G. Trahair^{1,2}, M. Horowitz^{1,2}, T. Hausken³, C. Feinle-Bisset^{1,2}, C.K. Rayner^{1,2}, K.L. Jones^{1,2};¹Discipline of Medicine, The University of Adelaide, ²NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, The University of Adelaide, Australia, ³Section for Gastroenterology, Department of Clinical Medicine, University of Bergen, Norway.

Background and aims: A postprandial fall in blood pressure (BP) occurs frequently in healthy older subjects and type 2 patients, the magnitude of which is related to the rate of gastric emptying (GE) and rise in splanchnic blood flow. Postprandial hypotension (PPH) is now recognised as an important clinical problem in these groups. In humans, exogenous glucagon-like peptide-1 (GLP-1) has been reported to increase BP in two studies, but not another, while clinical trials of GLP-1 agonists in type 2 diabetes and obesity have reported a modest reduction in BP and a rise in heart rate (HR). Studies relating to the cardiovascular effects of GLP-1 and its agonists have not, however, discriminated between fasting, as opposed to postprandial, BP and HR. The aims of this study were to determine whether exogenous GLP-1 modulates the effects of an intraduodenal (ID) glucose infusion on BP, HR and splanchnic blood flow in healthy older subjects.

Materials and methods: On two separate days, 10 healthy 'older' subjects (9M,1F; age 73 ± 2 yr) received an infusion of GLP-1 (0.9 pmol/kg/min IV), or saline for 90 min ($t = -30 - 60$ min) in random order. Between $t = 0 - 60$ min, ID glucose was infused at 3 kcal/min. BP and HR were assessed with an automated device (Dinamap, GE Medical Systems), superior mesenteric artery (SMA) flow by Doppler ultrasonography and blood glucose and serum insulin were also measured. Results are shown (mean \pm SEM).

Results: During the 'fasting' period ($t = -30 - 0$ min), GLP-1 had no effect on BP ($P = 0.40$) or HR ($P = 0.59$). In response to ID glucose ($t = 0 - 60$ min), systolic BP decreased ($P < 0.001$; Figure 1A), and both HR ($P < 0.001$; Figure 1B) and SMA flow ($P < 0.05$; Figure 1C) increased, on both days. GLP-1 attenuated the maximum fall in systolic BP (saline: -14 ± 3 vs. GLP-1: -9 ± 2 mmHg; $P < 0.05$), tended to increase the maximum rise in HR (saline: 13 ± 3 vs. GLP-1: 15 ± 3 BPM; $P = 0.09$) and increased the maximum rise in SMA flow (saline: 500 ± 52 vs. GLP-1: 837 ± 102 ml/min; $P < 0.01$). GLP-1 diminished the peak glycaemic response to ID glucose (saline: 10.3 ± 0.5 vs. GLP-1: 9.3 ± 0.3 mmol/L; $P < 0.05$) and increased the AUC $0 - 60$ min insulin/glucose ratio (saline: 3.5 ± 0.7 vs. GLP-1: 4.9 ± 0.9 ; $P < 0.05$).

Conclusion: In healthy older subjects, acute administration of GLP-1 attenuates the hypotensive response to ID glucose, apparently independent of changes in HR, and potentiates the increase in SMA flow. Given that GLP-1 also slows GE, a role in the management of PPH warrants evaluation.



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Impaired incretin effect and gastrointestinal-mediated glucose disposal in non-diabetic patients with cirrhosisF.K. Knop¹, L. Gluud², A.E. Junker¹, J.J. Holst³, T. Vilsbøll¹;¹Diabetes Research Division, Gentofte, ²Department of Gastroenterology, Hvidovre, ³Department of Biomedical Science, Copenhagen, Denmark.

Background and aims: Cirrhosis is often complicated by glucose intolerance, but the pathogenesis not yet completely understood. We evaluated the incretin effect and gastrointestinal-mediated glucose disposal (GIGD) in non-diabetic patients with biopsy-verified liver cirrhosis.

Materials and methods: Patients with compensated cirrhosis (Child Pugh A or B) and matched healthy controls underwent a 4h 50g-oral glucose tolerance test (OGTT) and an isoglycaemic intravenous glucose infusion (IIGI) on two separate days. We calculated the incretin effect [$100\% \times (\text{AUCC-peptide, OGTT} - \text{AUCC-peptide, IIGI} / \text{AUCC-peptide, OGTT})$], the GIGD

[$100\% \times (\text{glucose OGTT} - \text{glucose IIGI} / \text{glucose OGTT})$] and insulin resistance (according to the homeostatic model assessment (HOMAIR)). Characteristics of participants are summarised as median \pm interquartile range and compared using non-parametric analyses.

Results: Ten patients (5 women) with cirrhosis (age: 54 ± 15 years; BMI: 26 ± 6 kg/m²; fasting plasma glucose (FPG): 5.8 ± 0.7 mM; HbA1c: 38 ± 6 mmol/mol ($5.6 \pm 0.4\%$)) and 10 matched healthy controls (age: 58 ± 17 years; BMI: 29 ± 1 kg/m²; FPG: 5.2 ± 0.4 mM; HbA1c: 34 ± 6 mmol/mol ($5.5 \pm 0.2\%$)) were included. Patients with cirrhosis were more glucose intolerant (AUCOGTT: 609 ± 458 (cirrhosis) vs. 180 ± 109 mM \times min (controls), $P < 0.01$) and insulin resistant (HOMAIR: 3.7 ± 4.9 (cirrhosis) vs. 2.6 ± 1.4 (controls), $P < 0.05$) than controls. Isoglycaemia was achieved using 35 ± 12 g of glucose in patients with cirrhosis and 24 ± 10 g in healthy controls ($P < 0.01$). Patients with cirrhosis had impaired incretin effect (35 ± 44 (cirrhosis) vs. $55 \pm 30\%$ (controls), $P < 0.01$) and GIGD (30 ± 23 (cirrhosis) vs. $52 \pm 20\%$ (controls), $P < 0.01$).

Conclusion: Non-diabetic patients with cirrhosis exhibit impaired incretin effect and GIGD, which may contribute to the glucose intolerance associated with cirrhosis.

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Gastrointestinal-mediated glucose disposal in total pancreatectomised patientsA. Lund^{1,2}, J.I. Bagger^{1,2}, M. Christensen^{1,2}, M. Grøndahl^{1,2}, E.R. Mathiesen³, C.P. Hansen⁴, J. Storkholm⁴, S. Larsen¹, J.J. Holst², T. Vilsbøll¹, F.K. Knop^{1,2};¹Diabetes Research Center, Gentofte Hospital, ²Department of Biomedical Sciences, University of Copenhagen, ³Center for Pregnant Women with Diabetes, Department of Endocrinology, ⁴Department of Gastrointestinal Surgery, Rigshospitalet, Copenhagen, Denmark.

Background and aims: Gastrointestinal-mediated glucose-disposal (GIGD) after OGTT reflects the percentage of glucose disposal caused by the oral route of glucose administration. It accounts for as much as 70% in healthy subjects. Mediators of GIGD may include the incretin hormones, gut microbiota, first-pass hepatic uptake of glucose, and at present unknown factors. It is likely that incretin-mediated potentiation of pancreatic insulin secretion constitutes a major contributor to GIGD, but so far, it has not been possible to discriminate between pancreatic and extrapancreatic mechanisms underlying GIGD. We aimed to evaluate the impact of extrapancreatic effects on GIGD.

Materials and methods: Data from 7 total pancreatectomised patients (age: 61 ± 4 years; BMI: 22.3 ± 1.4 kg/m²; HbA1c: 63 ± 4 mmol/mol (mean \pm SEM)) and 8 healthy control subjects (age: 58 ± 3 years; BMI 23.2 ± 0.9 kg/m²; HbA1c: 32 ± 2 mmol/mol) were included in the present analysis. Participants were examined over two experimental days: a 75g-OGTT and a corresponding isoglycaemic i.v. glucose infusion (IIGI). Difference between administered glucose during OGTT and IIGI within the group of pancreatectomised patients were used to evaluate the impact of extrapancreatic mechanisms on GIGD.

Results: In healthy control subjects, 27 ± 2 g of glucose was infused intravenously to copy the plasma glucose profile from the 75g-OGTT, resulting in a GIGD of $65 \pm 2\%$. In total pancreatectomised patients we used 76 ± 3 g of glucose to copy the plasma glucose profile from the 75g-OGTT, resulting in a GIGD of $-2 \pm 5\%$ (i.e. that the pancreatectomised subjects disposed of i.v. and oral glucose similarly).

Conclusion: The low GIGD in pancreatectomised patients suggests that extrapancreatic factors do not play a major role in GIGD. The need for a larger glucose load on the IIGI day could however point to the existence of gut-derived factors (e.g. gut-derived glucagon) contributing to a worsening of oral glucose tolerance in pancreatectomised patients.

Clinical Trial Registration Number: NCT02006459

Supported by: EFSD/Novo Nordisk

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Preserved incretin effect despite significant hyperinsulinaemia following glucose loading in South Asians, in comparison to CaucasiansR. Ahluwalia¹, T.L. Vilsboll², L. Ranganath¹, J.J. Meier³, J. Vora¹, F.K. Knop²;¹The Royal Liverpool & Broadgreen University Hospitals NHS Trust, Liverpool, UK, ²Diabetes Research Division, Copenhagen University Hospital Gentofte, Denmark, ³Department of Medicine, St. Josef-Hospital, Ruhr-University, Bochum, Germany.

Background and aims: Circulating hyperinsulinaemia in normal glucose tolerant South Asians is reflective of underlying insulin resistance. Role of incretin hormones in this adaptive islet response in South Asians, remains to be explored. Our study aims to investigate the incretin effect in matched groups of South Asians and Caucasians.

Materials and methods: Ten South Asian subjects (age: 34 ± 4 years (mean \pm SEM); BMI 24.5 ± 1 kg/m²; fasting plasma glucose (FPG): 4.8 ± 0.2 mmol/l) and twelve age and BMI-matched Caucasian subjects (age: 31 ± 3 years; BMI 24.6 ± 1 kg/m²; FPG: 4.6 ± 0.1 mmol/l) with normal glucose tolerance were recruited. On two different days, subjects underwent a 4h 50g-OGTT and an isoglycaemic i.v. glucose (20%) infusion (IGII), respectively, involving sampling of insulin, C-peptide, glucagon and incretin hormones. The incretin effect was measured using the following formula based on beta cell secretory responses (AUCs): $100 \times (\text{AUC}_{\text{OGTT}} - \text{AUC}_{\text{IGII}}) / \text{AUC}_{\text{OGTT}}$.

Results: Fasting insulin levels were higher in South Asians vs. Caucasians (12.0 ± 1.6 vs. 8.0 ± 0.8 mU/l, $p=0.02$). The oral glucose curves were replicated successfully during the IGII studies. The integrated insulin responses after OGTT and IGII, respectively, were significantly higher in South Asians vs. Caucasians (OGTT: 10239 ± 1683 vs. 5377 ± 616 mU/l x min, $p=0.008$; IGII: 5610 ± 1347 vs. 2703 ± 321 mU/l x min, $p=0.03$). Incretin effects were similar in the two groups (48 ± 6 vs. $49 \pm 2\%$, $p=0.8$).

Conclusion: Our findings show preserved incretin effect despite insulin resistance and hyperinsulinaemia in normal glucose tolerant South Asians.

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Difference in insulin responsiveness between meal- and glucagon-loading test in Japanese type 2 diabetesT. Kitamoto^{1,2}, E. Nara², Y. Matsuzawa², J. Saito², M. Omura², T. Nishikawa²;¹Clinical Cell Biology and Medicine, Chiba University,²Endocrinology and Diabetes Center, Yokohama Rosai Hospital, Japan.

Background and aims: Responsiveness of c-peptide (CPR) to mixed meal tolerance test (MT) is a useful marker to evaluate the insulin secretion ability such as postprandial responsiveness of insulin production in type 2 diabetes. On the other hand, the total capacity of endogenous insulin secretion can be examined by the response of CPR after glucagon loading test (GT). Basal ability of insulin production was reported to be relatively impaired in Japanese type 2 diabetes, rather than Western patients, suggesting that incretin-related agents can be much more effective for glycemic control in Japanese than Western patients. Then, we tried to compare the responsiveness of CPR to MT and GT in Japanese type 2 diabetes for clarifying effectiveness of incretin-based therapy, such as DPP4 inhibitor (DPP4i) or GLP-1 analog (GLP-1A).

Materials and methods: We enrolled 95 Japanese type 2 diabetes patients prescribed DPP4i or GLP-1A from 2011 to 2012. CPR response (Δ CPR) to MT and GT was analyzed. Then, we divided the patients into 2 groups; controlled patients who reached glycemic target (HbA1c < 7.0%) and uncontrolled patients whose HbA1c was over 8.0% at 6 months after each treatment. We compared the clinical characteristics and responsiveness of CPR to MT with those to GT.

Results: DPP4i and GLP-1A were individually used in 47 and 48 patients, respectively. CPR response to MT was significantly higher in 29 cases than 7 uncontrolled patients among 47 patients treated with DPP4i (3.5 ± 2.0 vs. 1.9 ± 1.3 (ng/ml); $p=0.022$). CPR response to GT was significantly higher in 16 cases than 22 uncontrolled patients among 48 patients treated with GLP-1A (2.1 ± 0.8 vs. 1.3 ± 0.8 (ng/ml); $p=0.003$). The ROC analysis in each group showed optimal cut off point of Δ CPR by MT was under 2.0 ng/ml in cases showing HbA1c > 8.0% in DPP4i-treated group, while in GLP-1A-treated group, that of Δ CPR by GT was over 1.39 and under 1.26 ng/ml in cases showing HbA1c < 7.0% and HbA1c > 8.0%, respectively. Moreover, 86% of uncontrolled patients by DPP4i-treatment showed more than 1.39 in Δ CPR in GT, suggesting that they can be successfully treated with GLP-1A.

Conclusion: The present study clearly demonstrated that the gut-mediated insulin secretion estimated by MT can predict the efficacy of DPP4i, while total capacity of cAMP-mediated insulin secretion examined by GT may predict usefulness of GLP-1A. We can, therefore, simply decide whether DPP4i or GLP-1A might be effective for controlling hyperglycemia in Japanese type 2 diabetes by performing MT and GT.

PS 032 Novel approaches for identifying and targeting determinants of glucose tolerance

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Glypican-4 is increased in human subjects with impaired glucose tolerance and decreased in patients with newly diagnosed type 2 diabetes
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Background and aims: Glypican-4 (GPC-4), a membrane-bound hormone owing to a glycosyl-phosphatidylinositol (GPI) anchor, is synthesized and secreted by adipose tissues. GPC-4 differentially expressed in visceral and subcutaneous adipose tissue, and the expression in human white adipose tissue (WAT) was highly correlated with BMI and the WHR. However, no report has demonstrated the relationship of circulating levels of GPC-4 with impaired glucose tolerance (IGT) or T2DM in humans. The aim of the current study is to investigate whether GPC-4 correlates with obesity and insulin resistance by cross-sectional and interventional studies on anthropometric, metabolic, and hormonal predictors of circulating GPC-4.

Materials and methods: Circulating GPC-4 was measured with ELISA in subjects with NGT, IGT, and nT2DM who are involved in the study from June 1, 2013 to November 10, 2013. EHC were performed in healthy and T2DM subjects. Plasma glucose was measured by the glucose oxidase method. HbA1c was measured by HPLC. Plasma insulin was measured by radioimmunoassay. Plasma FFA was measured with a commercial kit. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were analyzed enzymatically using an autoanalyzer. Real-time RT-PCR and Western blotting were used to assess mRNA and protein expression of GPC-4.

Results: Circulating GPC-4 levels were significantly higher in IGT subjects and lower in nT2DM subjects compared to controls. Circulating GPC-4 was positively correlated with BMI, WHR, HOMA-IS and FAT%, while it was inversely correlated with FBG and HbA1c. Excluding diabetic subjects, increasing GPC-4 levels were associated with HOMA-IR and M values ($r = 0.247$ and $r = -0.491$; both $P < 0.05$). In T2DM patients, GPC-4 mRNA expression were significantly reduced by 35 % in muscle and 28 % in fat compared to normal controls (both $P < 0.05$). GPC-4 protein levels were significantly reduced by about 51 % in both muscle and fat relative to normal subjects (both $P < 0.05$). GPC-4 levels were significantly increased upon an oral glucose intake. The secretion of GPC-4 exhibited a characteristic diurnal rhythm in humans, with a major rise occurring between afternoon and midnight.

Conclusion: The present study revealed a difference in GPC-4 levels in normal, IGT and nT2DM subjects as well as a significant association between circulating GPC-4 levels and IGT or nT2DM and various anthropometric and metabolic parameters. Our results demonstrated for the first time that circulating GPC-4 concentrations were increased in IGT subjects and reduced in nT2DM subjects. We have also demonstrated that GPC-4 was associated with some metabolic or obesity related parameters, and that GPC-4 was affected by an oral glucose challenge. We also found evidence to suggest that the down-regulation of GPC-4 expression in muscle and adipose tissues may be responsible for the reduced circulating GPC-4 levels in T2DM patients. Thus, our data suggest that GPC-4 is likely to play a major role in the development of obesity, insulin resistance, and T2DM in humans.

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Bombesin receptor subtype-3 (BRS-3) and its synthetic agonist: therapeutic tandem for obese/type 2 diabetic patients

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Background and aims: BRS-3 gene/protein expressions are lower than normal (N) in skeletal muscle from obese (OB) or type 2 diabetic (T2D) patients; moreover in myocytes from all three groups, insulin-mimetic effects of [D-Tyr6,β-Ala11,Phe13,Nle14]bombesin6-14—synthetic BRS-3 agonist—, have been reported (v.g.: kinase phosphorylation increases, glucose metabolism improving). Also, our previous data related to BRS-3, in patients simultaneously diagnosed with obesity and type 2 diabetes (OB/T2D), pointed out its potential therapeutic interest, leading the current study in: A) pieces of skeletal muscle from OB/T2D—BRS-3 expression—; B) primary cultured myocytes from OB/T2D patients, —BRS-3 signalling pathways, by using [D-Tyr6,β-Ala11,Phe13,Nle14]bombesin6-14, and confirmed its effect on glucose transport (GT) and glycogen synthase activity (GSA)—.

Materials and methods: 10 OB/T2D patients (3F/7M; age:49±3 yrs; glucose:112±6 mg/dl; cholesterol:184±9 mg/dl; triglycerides:143±18 mg/dl; Dianben treatment) and 23 N subjects (19F/4M; age:50±3; glucose:96±2; cholesterol:185±8; triglycerides:98±9) undergoing surgery, previous informed consent given, were included. BRS-3 gene expression -RT-PCR-, BRS-3 protein, PKB, p70s6K, p42/44MAPKs and p90RSK1 phosphorylation -Western Blot-, GT and GSA -glucose precursors-; insulin acted as positive control. ANOVA (Levene/Bonferroni test; using SPSS 21.000) was performed; $p < 0.05$ significant value.

Results: In OB/T2D muscle pieces, BRS-3 mRNA level (23.6±1.3 times down-regulated, $p < 0.0001$; $n = 5$) and protein values (49±7% of N, $p < 0.05$; $n = 3$) were lower compared to N. In primary cultured myocytes from 5 OB/T2D, the phosphorylation basal values of all kinases were reduced ($p < 0.05$), compared to normal state. BRS-3's agonist at 10-11 up 10-8M, slightly increased PKB phosphorylation (overallmean:126±2% control, $p < 0.02$), being reduced compared to that exerted by insulin (10-9M:143±4%). At 10-10M ligand, p70s6K activation (136±10% control, $p = 0.035$), and 10-9M insulin (132±8%, $p = 0.037$) were equal; no increments compared to basal were detected at higher agonist concentrations (10-9 up 10-8M; overallmean: 109±4%). The ligand at 10-11M, induced maximal phosphorylation of MAPKs (p42:159±15% control; p44:166±11%; $p < 0.0001$), and p90RSK1 increase was showed at 10-11M compound (148±2%, $p < 0.0001$), being at 10-10 up 10-8M (overallmean:133±3%), identical to that detected at 10-9M insulin (133±4%). In OB/T2D myocytes, BRS-3-agonist stimulated GT, already detected at 10-12M (133±9% control), maximal at 10-11M (160±9%, $p < 0.0001$) and maintained up to 10-8M (overallmean: 153±7%, $p < 0.007$); this action was lower to that detected at 10-8M insulin (202±9%, $p = 0.009$). The effect of the agonist on GSA, achieved the maximum at 10-11M (165±16% control; $p < 0.0001$), which was not different, from that obtained at higher concentrations 10-10 up 10-8M (overallmean: 145±5%; $p < 0.008$).

Conclusion: Taken together these results from samples, which combine two major metabolic diseases -obesity and type 2 diabetes-, confirm that BRS-3 receptor and its agonist could be useful as a therapeutic tandem also for OB/T2D patients.

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Insight into the molecular mechanism of action of BTI320, a non-systemic novel drug to control serum glucose levels in individuals with diabetes

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Background and aims: Starch is an $\alpha(1\Rightarrow4)$ -linked polymer of glucose, which is enzymatically digested or hydrolyzed down to smaller polysaccharides (e.g. dextrin) and then onto smaller sugars like maltotriose and maltose, and eventually to glucose. The key enzymes responsible for the breakdown of starch

are generally called α -glycosidases, with α -amylase being primary among them. BTI320 is a novel, non-systemic therapy that safely reduces postprandial glucose excursions with reduced side effects compared with acarbose. Acarbose is a natural microbial pseudo-tetrasaccharide that binds reversibly and competitively to the oligosaccharide binding site of α -glucosidases. BTI320 is composed of non-glucose-containing polysaccharides. It is essentially a composite of two modified galactomannans: GM- α (1 \Rightarrow 1) linked polymer; and GM- β (1 \Rightarrow 4) linked polymer. We believe that BTI320 functions by targeting several polysaccharide hydrolyzing enzymes and that the active ingredient is GM- α .

Materials and methods: The present study was focused on assessing the molecular mechanism of action of BTI320 in relationship to α -amylase. We used ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy to investigate interactions between BTI320 components, GM- α and GM- β , and the enzyme α -amylase, as well as the effects that GM- α and GM- β have on the rates of amylase-mediated starch hydrolysis towards glucose. The amylose iodine assay was also used to assess starch hydrolysis. Results are compared with those on acarbose.

Results: Chemical shift changes in NMR spectra of α -amylase demonstrate that GM- α interacts with the enzyme, possibly at or near its active site; GM- β appears to have no effect on α -amylase. GM- α and GM- β both interact with starch and apparently change the amylose structure, thus affecting how amylase hydrolyses the starch. Under certain conditions, the rate constants for starch (1 mg/ml) hydrolysis with α -amylase goes from 22.5 s $^{-1}$ in the absence of GM- α to 2.7 s $^{-1}$ in the presence of 4 mg/ml GM- α ($p<0.005$). The effect on the rate of starch hydrolysis with acarbose is similar, but at acarbose concentrations of about 100-fold less. In addition, comparison of NMR data on α -amylase with GM- α and acarbose suggest that GM- α binds at or near the same site on the enzyme as acarbose.

Conclusion: GM- α acts as an inhibitor, possibly competitive, of α -amylase function, and appears to be the active ingredient in BTI320. Our findings provide insight into how BTI320 may function in vivo and support the potential viability of BTI320 as an alternative to acarbose therapy for glycemic control.

Supported by: Boston Therapeutics

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Different clinical responses to exenitide, insulin and pioglitazone are associated with TNF-857C/T polymorphism in newly diagnosed type 2 diabetic patients

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Background and aims: TNF- α is one of the pivotal proinflammatory cytokines contributing to insulin resistance. Yet the association of TNF-1031T/C-863C/A-857C/T genotype with clinical efficacy of exenitide, insulin or pioglitazone in type 2 diabetes has not been explored.

Materials and methods: Newly diagnosed and drug-naïve type 2 diabetic patients with HbA_{1c} between 7% and 10% were enrolled nationwide. All patients were randomly assigned to exenitide, insulin or pioglitazone group. TNF-1031T/C-863C/A-857C/T polymorphisms were obtained by genomic DNA sequencing. Clinical efficacy were evaluated by examining changes of HbA_{1c}, both fasting and 2 hour postprandial plasma glucose, insulin levels, HOMA-B, HOMA-IR, lipid profiles after one year treatment with anti-hyperglycemic agents mentioned as above and were analyzed according to different genotype groups. Molecular mechanisms of TNF polymorphisms on transcriptional regulation were investigated by constructing luciferase reporters carrying the corresponding single nuclear polymorphism followed by transient transfection in RAW264.7 cells and electrophoretic mobility shift assay.

Results: Ten different TNF-1031T/C-863C/A-857C/T genotypes were found in 382 enrolled patients. All baseline parameters were comparable among different TNF genotype groups. One year treatment of each agent effectively decreased HbA_{1c}, fasting and 2 hour postprandial plasma glucose levels ($P<0.05$). Weight gain was observed by the end of insulin therapy in all genotype groups, which resulting an increase of HOMA-IR accordingly ($P<0.05$). However, exenitide and pioglitazone reduced HOMA-IR significantly in patients with TNF-1031TT-863CC-857CC genotype (TCC)(before vs after: 1.58 \pm 0.62 vs 1.22 \pm 1.10, 1.68 \pm 0.63 vs 0.97 \pm 1.02, respectively, $P<0.05$). On the contrary, no statistically changes of HOMA-IR was induced in TNF-1031TT-863CC-857CT genotype carriers (TCT) by the same medications (1.77 \pm 0.50 vs 1.42 \pm 1.01, 1.49 \pm 0.78 vs 1.24 \pm 0.83). Exenitide induced a comparably dramatic weight loss as to 4.7% in TCC patients and 4.5% in TCT ones (both

$P<0.05$), waist and hip circumferences decreased in the former (before vs after, 89.95 \pm 8.25 vs 87.35 \pm 9.00, 98.08 \pm 5.35 vs 95.35 \pm 6.68 cm, respectively, $P<0.05$) while not in the latter (before vs after, 87.72 \pm 16.14 vs 86.81 \pm 10.45, 95.92 \pm 16.30 vs 96.00 \pm 7.06 cm, respectively). In vitro studies demonstrated that human TNF-857T-allele containing luciferase reporter exhibited over 2 fold greater transcriptional activity than that of the -857C allele across all experimental conditions: unstimulated or stimulated with LPS, IFN- γ , or both. EMSA analyses with biotin-labeled -857C- or T-allele probes revealed differential nuclear DNA-binding activities that were stronger with the C allele than the T allele, which transcriptional factor OCT1 could bind. And overexpression of OCT1 totally diminished differences of luciferase activity between -857T-allele and -857C-allele.

Conclusion: TNF-857T showed an OCT1 mediated, constitutively up-regulated transcriptional activities of TNF gene, which may restrain the effect of insulin resistance improvement of exenitide and pioglitazone in Chinese type 2 diabetic patients.

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The effect of atorvastatin vs pravastatin on glucose homeostasis in the Otsuka Long-Evans Tokushima Fatty (OLETF) rats

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Background and aims: New-onset diabetes has been observed in clinical trials and meta-analyses involving statin therapy. Controversy exists regarding the effects of statin treatment on progressive insulin resistance. To investigate the effect of atorvastatin (AS) vs pravastatin (PS) on glucose homeostasis in the Otsuka Long-Evans Tokushima Fatty (OLETF) rats.

Materials and methods: Thirty OLETF rats of 10 weeks of age were randomly divided into 3 groups: untreated (n=10); AS-treated (n=10); PS-treated (n=10) rats. As normal control rats (n=10), Long-Evans Tokushima Otsuka (LETO) were used. OLETF rats were either untreated or treated with AS (100 mg/kg per day) or PS (25 mg/kg per day) from 12 weeks of age for 24 weeks. We measured daily food intake, weekly body weight and casual blood glucose levels. At 20 and 36 weeks of age, an oral glucose tolerance test (OGTT) was performed from the tail vein after overnight fasting.

Results: Food intake and casual blood glucose levels did not show significant differences among OLETF rats during the periods of studies. However, compared with untreated OLETF rats, body weight was decreased significantly at 31 to 35 weeks of age in PS-treated rats. Long-term treatment with statins, either from 12 or 36 weeks of age, mean fasting blood glucose, insulin, GIP and glucagon levels were not statistically different among OLETF rats. However, fasting GLP-1 level at 20 weeks and stimulated 30 min GLP-1 level at 36 weeks were significantly low in statin treated OLETF rats compared with untreated OLETF rats ($p<0.002$), in addition, fasting C-peptide level at 36 weeks was significantly decreased in PS-treated OLETF rats compared with untreated OLETF rats, ($p<0.023$). Kitt, HOMA-IR and β -cell mass at 20 or 36 weeks of age were not significantly different between three treated OLETF rats compared with untreated OLETF rats.

Conclusion: The results suggest that long-term treatment with statin did not increased blood glucose levels, however, statin therapy might be associated with a decrease of GLP-1 levels over 20 weeks of age in statin treatment rat model of spontaneously developing type 2 DM. Taken together with previous reports, present study imply a role for affecting incretin physiology by statin.

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The identification of novel metabolites that track with improvements in glycaemia following a 12-week lifestyle intervention in high risk individuals

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Background and aims: A number of studies have identified fasting metabolites associated with insulin resistance, dysglycemia and type 2 diabetes. It is not known if metabolites respond to lifestyle modifications that reduce the risk of type 2 diabetes. The purpose of this study was to identify metabolite biomarkers, linked to dysglycaemia, that track with improvements in plasma glucose following a lifestyle intervention in high risk individuals.

Materials and methods: As part of the DEXLIFE programme, individuals with a FINDRISC score >12 or impaired fasting/2-hr glucose volunteered to participate in a 12-week diet and exercise intervention (n=104). An oral glucose tolerance test, body composition assessment (DEXA) and maximal aerobic capacity were determined before and after the intervention. Using the stable isotope dilution technique, quantitative assays were developed for a set of 23 candidate biomarker metabolites previously linked to dysglycemia. This set included: α -hydroxybutyric acid (AHB), linoleoylglycerophosphocholine (LGPC), oleic acid, α -ketoglutaric acid, 2-aminoadipic acid, glycine, aromatic amino acids, and the 3 branched-chain amino acids and several of their catabolites.

Results: After the 12-week intervention fasting levels of 12 of the 23 metabolites were significantly different (p10% (n=16) there was a significant decrease in plasma tyrosine, α -ketoglutarate and phenylalanine (p<0.05) as well as increased glycine and serine (p10% decrease in 2-hr glucose (n=35) was associated with significant decreases in branched-chain amino acid catabolites 3-methyl-2-oxopentanoic acid, 3-methyl-2-oxo-butyric acid, 4-methyl-2-oxopentanoic acid (p<0.05) in addition to insulin, α -ketoglutarate, tyrosine (p<0.05) and increased glycine (p<0.05). The fold change in body weight was positively associated with the fold change in phenylalanine, tyrosine, leucine, isoleucine, 3-methyl-2-oxopentanoic acid and insulin (p<0.05) and negatively associated with glycine (p<0.05).

Conclusion: A subset of metabolites linked to dysglycaemia track with improvements in fasting and 2-hr glucose following a 12-week lifestyle intervention in high risk individuals. These metabolites are sensitive to small changes in metabolic function and may be useful for monitoring diabetes prevention programmes.

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Fasting and postprandial hyperglycaemia: their relative contributions to the overall hyperglycaemia and their determinants in Korean patients with type 2 diabetes

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Background and aims: Monnier et al. showed greater contributions of postprandial (PHG) and fasting (FHG) hyperglycemia to overall hyperglycaemia at lower and higher HbA1c, respectively. However, it has never been studied in Korean diabetic patients. In addition, there have been few studies about determinant factors in postprandial and fasting glucose levels. Therefore, we assessed the relative contributions of PHG and FHG to the overall hyperglycemia and the influencing factors on PHG and FHG in Korean patients with type 2 diabetes.

Materials and methods: We enrolled 195 Korean type 2 diabetic patients which did not take insulin or α -glucosidase inhibitor. They performed a seven-point self-monitoring of blood glucose (7-point SMBG) more than once during each month for 3 consecutive months. Glucose area under the curve (AUC) above 100 mg/dL (5.5 mmol/L) was defined as AUC(total) to represent the overall hyperglycemia. The area under the curve above fasting glucose level was considered the postprandial hyperglycemia (AUC(PHG)).

The fasting hyperglycemia (AUC(FHG)) was calculated as [(AUC(total) - AUC(PHG))]. The relative contributions of PHG and FHG to overall hyperglycaemia were respectively defined as the proportions of AUC(PHG) and AUC(FHG) to AUC(TOTAL).

Results: The relative contribution of PHG showed a significant difference and gradual decrement according to increasing quartiles of HbA1c (66.9±6.0, 36.1±6.0, 40.3±3.2, 32.3±3.8%; P(ANOVA)<0.001, P(TREND)<0.001). And the contribution of FHG was increased progressively with increasing quartiles of HbA1c (33.0±9.5, 63.8±6.0, 59.6±3.2, 67.6±3.8%; P(ANOVA)<0.001, P(TREND)<0.001). AUC(PHG) was positively correlated to age (r=0.191; p<0.01), systolic blood pressure (r=0.185; p<0.01), duration of diabetes age (r=0.185; p<0.01), C-peptide (r=0.198; p<0.01), HbA1c (r=0.282; p<0.01), and hsCRP (r=0.145; p<0.05). AUC(FHG) was positively correlated to body weight (r=0.190; p<0.01), waist circumference (r=0.185; p<0.01), C-peptide (r=0.217; p<0.01), HbA1c (r=0.658; p<0.01), alanine aminotransferase (r=0.228; p<0.01), and triglyceride (r=0.278; p<0.01) but negatively correlated to age (r=-0.146; p<0.05). Using multiple linear regression to adjust for age, sex and other covariates, only age (β =0.181; p<0.05) and triglyceride (β =0.150; p<0.01) remained significant variables of the AUC(PHG) and AUC(FHG), respectively.

Conclusion: In Korean type 2 diabetic patients, postprandial hyperglycemia predominantly contribute to overall hyperglycemia at lower HbA1c level, whereas fasting hyperglycemia is a predominant contributor to it at higher HbA1c level. And age and plasma triglyceride are independent predictors of postprandial and fasting hyperglycemiae, respectively.

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A putative role of P4 and E2 in the maternal late-onset glucose intolerance induced by gestational glucocorticoid excess

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Background and aims: In order to guarantee a proper maternal glycaemic control, at the end of pregnancy the pancreatic islets undergo a transient morpho-functional remodelling. We have shown that pancreatic beta cell resetting involves the modulation of several intracellular pathways, most of them in a glucocorticoid (GC)-dependent manner. On the other hand, we have recently described that the excess of GC at the end of pregnancy results in a late-onset of glucose intolerance due to an impairment of GSIS. This phenomenon is correlated with an early up-regulation of miR-29 expression, a validated target of progesterone (P4). Of note, P4 was reported as a beta cell pro-apoptotic hormone, whereas estrogen (E2) abolishes the cytokine-induced beta-cell death. Thus, we hypothesized that the functional resetting of the maternal pancreatic islets could depend on the proper E2 and P4 signalling. The aim of this work was to investigate the mechanisms involved in the late-onset maternal glucose intolerance induced by gestational GC excess, focusing on E2 and P4 signalling in pancreatic islets.

Materials and methods: Pregnant Wistar rats were treated with dexamethasone, 0.2 mg/kg daily in drinking water during the 14th to 19th day of pregnancy (P-DEX). Age-matched untreated pregnant rats (P) were used as controls. At the 19th day of pregnancy, DEX-treated and untreated rats were euthanized and pancreatic islets were isolated and used for western blot, qPCR analysis and GSIS; serum was used for P4 and E2 measurement. The Ethics Committee approved the study, and complies with the Brazilian Society of Laboratory Animal Science.

Results: P4 levels were significantly reduced in P-DEX compared to P rats (respectively 155.7±45.1 and 295.5±44.8 ng/ml; p<0.05), as well as the expression of progesterone receptor (PR) (0.2±0.1 times of P group; p<0.05). Neither E2 levels (55.6±22.6 and 41.4±20.8 pg/ml, respectively for P and P-DEX rats) nor ER β expression was altered by the treatment, but ER β was increased (9.4±2.6 times higher than P islets; p<0.05). SGK1, a GC target that negatively regulates insulin secretion, was upregulated in islets from P-DEX rats (1.9±1.5 times of control values; p<0.05). In parallel, maximum insulin response to glucose was lower than untreated P rats (0.6±0.2 times of P; p<0.05), but still responsive to glucose. In the presence of 2.8 mM glucose, insulin secretion values were 0.53±0.06 ng/ml (0.48±0.04 ng/ml in virgin rats), and with 16.7 mM glucose the values reached 1.17±0.19 ng/ml (1.34±0.10 ng/ml in virgin rats). The expression of inflammatory markers was also assessed. Compared to P islets, phospho-IKK/IKK ratio and NF- κ B expression were reduced in P-DEX (respectively 0.7±0.1 and 0.3±0.1 times of P islets; p<0.05).

Conclusion: The lower levels of P4 and PR in P-DEX islets is likely to be a factor involved in the previously described up-regulation of miR-29. The up-regulation of ER β induced by DEX treatment could be preserving the secretory responsiveness to glucose. Finally, the down-regulation of inflammatory markers could disrupt beta cell resetting after parturition, thus reflecting in an inappropriate maternal glucose homeostasis.

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PS 033 Determinants of insulin sensitivity and glycaemic control

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Determinants of insulin sensitivity in Middle Eastern immigrants and native Swedes

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Background and aims: Little is known about the metabolic, lifestyle and heritable risk factors that underlie the observation that type 2 diabetes (T2D) is highly prevalent amongst Middle Eastern immigrants to Northern Europe. Hence, in the current report from the MEDIM study (the impact of Migration and Ethnicity on Diabetes in Malmö), we aimed to (1) investigate insulin sensitivity and secretion (2) study risk factors associated with T2D and (3) investigate the contribution of metabolic-, demographic- and lifestyle-related risk factors for impaired insulin sensitivity index (ISI) in immigrants from Iraq, with comparisons to native Swedes.

Materials and methods: Population-based, cross-sectional study conducted 2010–2012 including residents 30 to 75 years of age born in Iraq or Sweden, in whom 10-h fasting blood samples and oral glucose tolerance tests were performed. Further, sociodemography, lifestyle behaviors and comorbidity were assessed by questionnaires. Insulin sensitivity and secretion were assessed by Matsuda indices. Outcomes were T2D and ISI. Associations were assessed by logistic- and linear regression analysis.

Results: In Iraqi ($n=1398$) compared to Swedish ($n=757$) participants, T2D was twice as prevalent (11.6 vs. 5.8%, $p<0.001$) and T2D onset occurred more than six years earlier. The prevalence of family history of T2D was twice as frequent in Iraqis compared with Swedes. ISI was generally lower in Iraqis as compared to Swedes (ISI 76.9 vs. 102.3, $p<0.001$ (mmol/L*mIE/L)⁻¹). Although insulin secretion (CIR) was generally higher in Iraqis, insulin response relative to the degree of insulin resistance (DIO) was lower in Iraqis than in Swedes (12712.9 vs. 14659.2 mmol/L⁻¹* mmol/L⁻¹ mmol/L⁻¹, $p<0.001$). Lower ISI and DIO in Iraqis than in Swedes were also observed in participants without T2D as well as in participants with normal waist circumference and/or normal weight (BMI<25kg/m²). Further, Iraqis without T2D presented a less advantageous glucose and lipid profile, larger waist circumference and higher BMI compared to Swedes (Table 1). Irrespective of other traditional risk factors for T2D, ISI conveyed higher standardized odds of T2D in Iraqis than in Swedes; the difference between the magnitude of these relationships was confirmed by a statistically significant interaction between country of birth and ISI ($P_{\text{interaction}}=0.044$). There was no interaction between country of birth and CIR. The crude difference in ISI according to ethnicity remained significant even when adjusting for metabolic-, anthropometric-, and lifestyle factors.

Conclusion: The key findings of this study are that there are ethnic differences in the relationship of insulin sensitivity with T2D; our study suggests that ethnic background modifies the effect of insulin sensitivity on the risk of T2D diabetes and further that the impaired insulin sensitivity in this Arabic population is only partly explained by excess waist circumference or other known risk factors for insulin sensitivity. We conclude that Middle Eastern immigrant populations are likely to benefit greatly from interventions focused on improving insulin sensitivity.

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Effect of lowering plasma glucose and FFA concentrations on insulin sensitivity and beta cell function in T2DM individuals

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Background and aims: Chronic elevation in plasma FFA (lipotoxicity) and plasma glucose (gluco-toxicity) concentrations have been shown to have detrimental effect on both core defects (insulin resistance and beta cell dys-

function) in T2DM. We have shown that lowering plasma FFA and plasma glucose concentrations separately in T2DM individuals improves beta cell function and ameliorate insulin resistance. In the present study, we examined the effect of lowering both the plasma FFA and glucose concentrations on beta cell function and insulin sensitivity in T2DM individuals.

Materials and methods: 12 T2DM subjects (age=49 ±3, BMI=31.1± 1.8, HbA1c = 8.5 ± 0.3, diabetes duration = 8.5 ± 0.3 years) received treatment with dapagliflozin (10 mg/day) for 3 weeks and during the third week they also received acipimox (250 mg four times daily). 75 gram OGTT and euglycemic insulin clamp were performed at baseline and at weeks 2 and 3.

Results: Compared to baseline, dapagliflozin lowered the fasting plasma glucose concentration at 2 weeks (181±9 to 146±9, $p<0.01$); the decrease in (FPG) was accompanied by significant increase in insulin-stimulated total glucose disposal (TGD) (4.16 ±0.71 to 5.15±0.73, $p<0.05$), and beta cell function. $\Delta C\text{-pep}/\Delta G_{[0-120]}/IR$ increased by 105% ($p=0.003$). At week 3, plasma FFA concentration was decreased by 28% compared to week 2 and this decrease was accompanied by a small, non-significant increase (by 9%) in insulin-mediated TGD, beta cell function ($\Delta C\text{-pep}/\Delta G_{[0-120]}/IR$) increased further by 53% (0.15±0.03 to 0.23 ±0.04, $p=0.02$).

Conclusion: Simultaneously lowering the plasma glucose and FFA concentrations has an additive effect to improve beta cell function, but not to improve insulin sensitivity.

Supported by: NIH

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Effects of a lifestyle intervention in a pre-diabetic mouse model: glucose homeostasis and pancreatic beta cell function and mass

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Background and aims: Pancreatic beta-cells play a major role in obesity-related type 2 diabetes (T2DM) aetiology. Thus, pancreatic beta-cell plasticity is defined as the ability of the cells to adapt their function and mass to the variations in insulin demand. It is already known that during pregnancy mouse pancreatic beta-cell mass increases but it is then rapidly reversed towards the end of the pregnancy. Although pancreatic beta-cell mass has been extensively studied, many considerations of its plasticity in different physiological and pathological situations remain still unknown. This study assesses the adaptations that occur in pancreatic beta-cells before and after following a lifestyle intervention programme aimed to recover glucose homeostasis in an animal model of diet-induced obesity and pre-diabetes.

Materials and methods: C57BL/6J male mice were divided in three groups: a control group (Ctrl) (fed with chow standard diet), a pathological group (HFD) (fed for 16 weeks with 45% HFD, D12451 Open Source DietsTM) and an intervention group (Int) in which mice followed a lifestyle intervention after they have reached the pathological state. This lifestyle intervention consisted on calorie restriction, modification of the 45% HFD with mono- and poly-unsaturated fatty acids and the replacement of sucrose by corn starch, and an exercise training programme for 5 weeks. Insulin tolerance test (ITT), glucose tolerance test (IGTT), body weight evolution, fat volume, tissue weights, inflammatory profile, and fasting insulin and leptin levels were assessed for the three groups. Pancreases were excised for immunohistochemistry studies. Pancreatic islets were isolated for in vitro glucose-stimulated insulin secretion (GSIS).

Results: The mice in the HFD group were glucose intolerant and showed insulin resistance when compared to their littermates, Ctrl group. Moreover, those mice on the HFD group were significantly overweight, hyperglycemic, hyperinsulinemic, and hyperleptinemic after an overnight fasting. Morphological analyses of the pancreases showed that the HFD group mice had an increase in beta-cell mass with more and bigger pancreatic islets than the Ctrl littermate mice. Isolated islets from HFD mice showed an increase in in vitro GSIS when incubated in 16.7mM of glucose. The mice on the Int group were leaner, returning to the Ctrl group body, liver and pancreas weight values and reducing the increase in fat volume of the mice in the HFD group. Those mice showed a general improvement in glucose homeostasis. However, the mice on the Int group were still moderately hyperglycemic and hyperinsulinemic after overnight fasting. Morphometric analysis of the pancreas showed that insulin positive area remained unchanged with respect to the HFD group although a significant increment in the number of small islets was observed.

Conclusion: Our lifestyle intervention improved most of the deleterious effects that led to a diet-induced pre-diabetic state in the absence of significant effects on pancreatic beta cells.

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Plasma 26Rfa is increased in diabetic patients: evidence for an involvement in glucose homeostasis

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Background and aims: 26Rfa is an orexigenic neuropeptide identified as the endogenous ligand of the human orphan receptor GPR103. Moreover, animal studies suggest that 26Rfa is involved in the pathophysiology of obesity. The aim of this study was to investigate plasma 26Rfa level in obese and diabetic patients, and to explore a possible link between 26Rfa and glucose homeostasis.

Materials and methods: Fasting plasma 26Rfa was determined by using a specific radioimmunoassay in 161 subjects divided into 4 groups: controls, obese patients, obese type 2 diabetic patients and type 1 diabetic patients. Plasma 26Rfa was also evaluated during a 180 min oral glucose tolerant test (OGTT) in 10 healthy controls. Immunohistochemical studies with anti-26Rfa and anti-GPR103 antibodies were performed on human pancreatic slices. Finally, 26Rfa was injected in C57Bl mice during an intraperitoneal glucose tolerance test (IPGTT).

Results: Fasting plasma 26Rfa was significantly increased in obese patients as compared to controls (352±47 vs 224±32 pg/ml; $p<0.05$), but even more in type 1 and type 2 diabetic patients (605±115 pg/ml and 677±99 pg/ml vs controls; $p<0.001$). Plasma 26Rfa was positively correlated with age and blood glucose, but not with the BMI. In multivariate linear analysis, the correlation between fasting 26Rfa level and blood glucose was independent from age. During the OGTT in controls, plasma 26Rfa significantly increased at 120 min after the oral glucose load (+57,3%; $p<0.05$). Immunohistochemical studies revealed that 26Rfa and its receptor were present in the beta pancreatic cells. Finally, intraperitoneal 26Rfa injection during IPGTT was associated with a reduced glucose-induced hyperglycemia, and an increase of insulin secretion.

Conclusion: This study reports for the first time that plasma 26Rfa is increased in diabetic patients and is correlated to blood glucose. Moreover, animal studies suggest that the neuropeptide exerts its anti-hyperglycemic effect, at least in part, via an increase of insulin secretion.

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An unusual metabolic interplay: type 1 diabetes and glutaric aciduria type 1

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Background and aims: Type 1 diabetes (T1D) metabolic balance is affected by several variables (foods, insulin dose and daily-life activities of our patients). The management becomes more difficult if an extra factor affects the glucose homeostasis such as in the case we report: the concomitant occurrence of Glutaric aciduria type 1 (GA1, a rare organic aciduria) and Type 1 diabetes in a young boy. Even if their coexistence may appear a pure coincidental association, their interplay shows an unexpected phenotype.

Materials and methods: A 21 months boy, treated for T1D since the age of 15 months (anti-GAD>2000kU/L at onset), was referred to our attention from another center for the onset of GA1. Due to the severe neurological impairment, consequent on GA1 related basal ganglia damage, he required enteral nutrition via nasogastric tube, receiving an age adapted lysine free diet, arginine (100 mg/kg/die) and carnitine (160 mg/kg/die). Insulin therapy was adapted to this regimen. CGMS (Medtronic®Guardian RT) highlighted a marked tendency to hypoglycemic events (30% of daily values below the range (mean AUC<3.9mmol/L: 0,8) in the early morning hours (4-6am), poor controlled by our insulin adjustments. (fig.1;human insulin, blue arrows; glargine, red arrow).

Results: After 15 days from the beginning of diet and therapy for GA1 we observed a drop of anti GAD to 233KUI/L and a marked reduction of hypoglycemia (mean AUC<3.9mmol/L: 0), with an increased insulin requirement.

Conclusion: Animal models of GA1 showed an impairment of gluconeogenesis, involving Krebs disfunction in liver and GAD enzyme in brain. To date it has never been confirmed in humans with GA1. This is the first CGMS data from a GA1 patient: the hypoglycemia during nocturnal starvation reflects this gluconeogenetic impairment. The reduction of hypoglycemic events, the drop of anti-GAD, with the beginning of GA1 therapy, supports the observation on animal models. We can suppose that the contemporary presence of anti-GAD and the deficit of Krebs in T1D, may exacerbate the hypoglycemic events of GA1 making them clinically relevant.



Supported by: Medtronic provided CGMS device.

PS 033 Determinants of insulin sensitivity and glycaemic control

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Higher mean glucose levels and glucose variability in non-diabetes sepsis/septic shock patients than in healthy subjects

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Background and aims: Hyperglycemia is one of the factors linked to increase mortality in sepsis or septic shock patients. Retrospective study had shown that the glucose variability (GV) associated with increased mortality in these patients. Until now, data on GV in sepsis/septic shock patients are sparse. Moreover, there is no prospective study to demonstrate whether glucose variability in sepsis patients is different from healthy subjects. Therefore, the aims of this study were to compare glucose profiles including mean glucose levels and glucose variability between sepsis patients and healthy subjects, and to study the correlation of glucose variability calculated from glucose level obtained from continuous glucose monitoring (CGM) and capillary plasma glucose (CPG).

Materials and methods: Sepsis patients (SEPSIS) who had no history of diabetes and hemoglobin A1c (A1C) < 6.5% were recruited. All subjects had 72-hour continuous glucose monitor or CGM, using iPro 2 (Medtronic, US) within 12 hours after admission. 72-hour CGM was also applied to healthy subjects without diabetes to serve as a control group (CON). Capillary plasma glucose (CPG) was obtained from both groups for 4 to 6 times per day during the study period 3. Glucose levels (GL) obtained from CGM and CPG during the 24-hour and 72-hour were used to calculate mean glucose level (MGL24h and MGL72h), and glucose variability including standard deviation (SD24h and SD72h), coefficient of variation (CV24h and CV72h) and mean amplitude of glycemic excursion (MAGE24h and MAGE72h).

Results: This was a prospective study. Fourteen subjects with sepsis and ten healthy subjects were recruited. SEPSIS was older than CON whereas there was no difference in sex, BMI and A1C. Glucose profiles obtained from CGM and CPG demonstrated the similar results as shown in table. The MGL24h and MGL72h were significant higher in SEPSIS than CON. The GV during 72-hour as demonstrated by SD72h, MAGE72h were also higher in SEPSIS than CON. The glucose profiles obtained from CGM and CPG in SEPSIS had a strong correlation.

Conclusion: Patients without diabetes who sepsis had elevated mean glucose levels at 24-hour and 72-hour and glucose variability at 72-hour as compared to healthy subjects. Therefore, the strategy aimed to target both mean glucose level and glucose variability might have benefit in non-diabetic patients with sepsis/septic shock. We also demonstrated that the use of 4 to 6 time points of capillary glucose levels to calculate the glucose profiles provided accurate data similar to those obtained from CGM in sepsis patients.

Table 1: Glucose profiles from CGM and CPG in SEPSIS and CON during 24 and 72 hours

	Continuous glucose monitoring (CGM)			Capillary plasma glucose (CPG)		
	Sepsis (n=14)	Control (n=10)	P value	Sepsis (n=14)	Control (n=10)	P value
24 hours						
MGL _{24h} (mg/dl)	156.7 ± 30.7	97.4 ± 9.0	<0.001	159.9 ± 34.7	96.5 ± 8.1	<0.001
SD _{24h}	18.2 (9.5, 58.2)	14.2 (9.7, 21.0)	0.178	26.6 (9.5, 68.0)	14.5 (6.7, 42.5)	0.020
CV _{24h}	13.1 (6.9, 42.2)	16.1 (9.7, 21.9)	0.891	16.3 (6.9, 42.2)	14.8 (8.1, 47.0)	0.700
MAGE _{24h} (mg/dl)	57.2 (28.7, 179.1)	44.2 (33.7, 60.7)	0.178	-	-	-
72 hours						
MGL _{72h} (mg/dl)	145.5 ± 24.5	99.3 ± 6.8	<0.001	150.2 ± 28.5	98.1 ± 6.7	<0.001
SD _{72h}	22.1 (9.1, 61.1)	14.3 (7.9, 20.7)	0.026	29.0 (10.5, 61.1)	16.6 (9.3, 28.6)	0.017
CV _{72h}	15.2 (6.9, 42.9)	14.4 (8.1, 21.7)	0.756	16.6 (7.8, 42.9)	18.0 (8.9, 29.2)	0.474
MAGE _{72h} (mg/dl)	63.4 (31.0, 176.6)	46.6 (25.5, 62.6)	0.041	-	-	-

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Effects of chronic variable stress and dietary fat on insulin sensitivity

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Background and aims: Stress is a state of threat to homeostasis that may contribute to metabolic disorders such as visceral obesity and type 2 diabetes. However, there is a lack of studies analyzing the long-term consequences of chronic stress on insulin sensitivity and its relation to fat consumption. Our aim was to characterize how chronic variable stress (CVS) in relation to dietary fat affects insulin sensitivity in mice.

Materials and methods: Three-months old, body weight (BW)-matched male C57BL/6 mice were exposed to a random series of stressors for 15 days (CVS), the unstressed control mice were housed separately (Ctrl). Body composition was analyzed with NMR. After CVS, blood was collected (08:00–12:00 am) for corticosterone and insulin analysis. Subsequently, mice were consuming a low- (Chow) or a high-fat diet (HFD) for three months and insulin sensitivity was measured *in vivo* with hyperinsulinemic-euglycemic clamps. Statistical differences were considered significant at $p < 0.05$ (two-tailed unpaired t-test).

Results: CVS mice ($n=24$) had lower BW (25.49 ± 0.33 g, $p < 0.001$) and lean mass (23.08 ± 0.37 g, $p < 0.001$) with no changes in fat mass (2.23 ± 0.08 g) compared to the Ctrl group ($n=24$), (28.32 ± 0.36 g, 25.37 ± 0.39 g, 2.25 ± 0.09 g, respectively). Plasma levels of corticosterone were higher in the CVS group ($n=22-24$) (109.90 ± 13.71 vs 60.37 ± 7.55 ng/ml, $p < 0.01$) as well as insulin levels (0.65 ± 0.06 vs 0.47 ± 0.03 ng/ml, $p < 0.01$) compared to the Ctrl group ($n=19-24$). When fed Chow, the CVS group ($n=10$) showed a trend to lower basal endogenous glucose production (EGP) (20.01 ± 1.12 vs 23.86 ± 1.48 mg/kg/min, $p=0.055$), while insulin-stimulated glucose disposal (Rd) in peripheral tissues was increased in CVS compared to Ctrl (363 ± 29 vs 275 ± 17 % of basal, $p < 0.05$). On HFD, basal EGP was unchanged, while Rd (135 ± 12 vs 192 ± 14 % of basal, $p < 0.01$) was decreased with CVS. Suppression of EGP by insulin was unaffected by CVS. CVS-Chow mice showed lower plasma adiponectin (5.87 ± 0.43 vs 11.29 ± 1.39 μ g/ml, $p < 0.01$) and a trend towards lower resistin (0.13 ± 0.005 vs 0.14 ± 0.005 μ g/ml, $p=0.06$) compared to Ctrl. CVS-HFD mice showed lower plasma adiponectin (7.99 ± 0.89 vs 15.74 ± 3.28 μ g/ml, $p < 0.05$) and a trend towards higher resistin (0.17 ± 0.02 vs 0.11 ± 0.02 μ g/ml, $p=0.06$) compared to Ctrl.

Conclusion: When fat consumption is low, chronic stress improves insulin sensitivity through higher peripheral glucose disposal. On the contrary, HFD consumption exacerbates insulin resistance under these conditions. Changes in adipokine profiles could be a possible mechanism underlying the effects of CVS and dietary fat on peripheral insulin sensitivity.

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Oxygen restriction as challenge test reveals early diet-induced health effects

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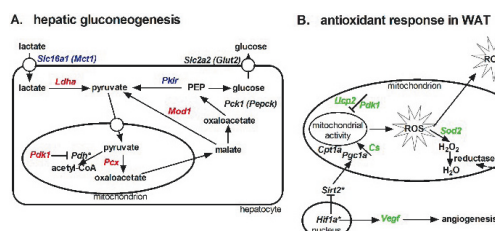
Background and aims: Challenge tests stress homeostasis and may reveal deviations in health that remain masked under unchallenged conditions. Ideally, challenge tests are non-invasive and applicable in an early phase of a study. The response to oxygen restriction (OxR; mild normobaric 12% O₂) measures the flexibility to adapt metabolism. Metabolic inflexibility is one of the hallmarks of the metabolic syndrome and underlying insulin resistance.

Materials and methods: Male C57BL/6J OlaHsd adult mice were fed a low-fat (LF) or high-fat (HF) diet for only five days. Indirect calorimetry was used to assess the response to OxR. Serum markers, including protein glycation/oxidation, and gene expression in liver and white adipose tissue (WAT) were analyzed.

Results: Although HF-fed mice had a significant higher body weight (BW) after five days of feeding, HF-fed and LF-fed mice did not differ in calorimetric values under normal conditions nor in fasting state. Moreover, the

subgroups of mice that were fasted and exposed to OxR during the last 6h showed no differences in BW (gain), nor in blood glucose levels. Exposure to OxR, however, revealed significant differences in substrate use with the HF-fed mice showing higher fatty acid oxidation levels and oxygen consumption, while their activity was similar to LF-fed mice. Furthermore, hepatic and WAT transcript levels differed significantly between both groups indicating differences in their adaptation to OxR. Only HF-fed mice showed increased hepatic lactate/glucose metabolism upon OxR, while LF-fed mice showed an increased oxidative stress response in WAT (see Figure). The adaptation in HF-fed mice appeared to be dampened, associated with increased serum markers of protein glycation/oxidation, whereas these changes were absent in LF-fed mice.

Conclusion: An oxygen restriction challenge test is a promising new method to test food products on potential beneficial effects for metabolic health. Differential metabolic adaptations upon OxR in hepatic lactate metabolism and stress response. Transcripts appeared up-regulated by OxR both in LF and HF (blue), only in HF (red), only in LF (green), or non-regulated (black). Lactate/Glucose metabolism (A) was analysed in liver, stress response mainly in WAT (B). Underlined transcripts denote up-regulation by HF vs LF under normoxia. Mod1 appeared down regulated by HF vs LF under normoxia. *: not determined.



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A syndrome of hyperinsulinaemic reactive hypoglycaemia associated with mutations in the human insulin receptor gene: two cases report

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Background and aims: Hyperinsulinemic hypoglycaemia in adults is the most frequently caused insulinoma but some cases, especially reactive hypoglycaemia, remain unexplained. We have experienced two independent cases of hyperinsulinemic reactive hypoglycaemia with adolescence or adult onset and analysed their pathophysiology and insulin receptor (IR) genes including those of their relatives.

Materials and methods: Genomic DNA was extracted from the patient's leukocytes and the direct sequencing for all exons of IR gene was performed. In overexpression experiments, we used pCMV expression vector and HEK293 cells, and the mutant IR described below was generated by PCR-based mutagenesis.

Results: Case 1 was a 42-year-old man. He had experienced recurrent episodes of finger tremor and cold sweat that dispelled by drinking juice since he was about 30 years old. His body mass index was 20.7. Laboratory tests showed fasting plasma glucose (FPG) was 76 mg/dl, fasting immunoreactive insulin (F-IRI) was 19.0 μ U/ml, fasting C-peptide (F-CPR) was 1.37 ng/ml, and HbA1c was 5.0%. A 75g OGTT exhibited normal glucose tolerance pattern and reactive hypoglycaemia at 3h. Case 2 was a 34-year-old man. Since he was fifteen years old, he had experienced recurrent episodes of finger tremor and cold sweat that dispelled by drinking juice. Laboratory tests showed FPG was 104mg/dl, F-IRI was 71.7 μ U/ml, F-CPR was 3.1ng/ml, and HbA1c was 6.7%. A 75g OGTT exhibited diabetic pattern and reactive hypoglycaemia at 4h. In both cases, neither anti-insulin nor anti-IR antibody was observed. We found that the former case had a novel missense mutation (Arg229Cys) in both alleles of IR gene, and both his parents had the same mutation only in one allele but not hyperinsulinaemic reactive hypoglycaemia. Furthermore, we also found that the latter case had a novel nonsense mutation (Trp1273X) followed by a mutation (Gln1274Lys) in a single allele of IR gene and his nine-year old son had the same mutation in one allele and also hyperinsulinaemic reactive hypoglycaemia. Overexpression experiments of the mutant gene (Arg229Cys) in HEK293 cells revealed increase of proreceptor-mature

receptor ratio of IR protein and reduced Akt phosphorylation by insulin stimulation (at concentration of 0.1 or 1.0nM) compared to wild type.

Conclusion: In two cases of hyperinsulinaemic reactive hypoglycaemia, we found novel mutations in the IR gene considered to be linked to their hypoglycemia. We propose a disease entity of a syndrome of hyperinsulinaemic reactive hypoglycaemia associated with mutations in the human IR gene.

PS 034 Glucose uptake and glucose action

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APPL1 affects on glucose uptake induced by uniaxial stretch in C2C12 myotubes

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Background and aims: Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1 (APPL1) was identified as protein kinase B or Akt (PKB/Akt) binding protein. It has been reported that APPL1 highly expresses in adipose and muscle tissues and has an important role in GLUT4 translocation in response to insulin and adiponectin via Akt or 5' adenosine monophosphate-activated protein kinase (AMPK) dependent pathway. We found that Akt and AMPK were activated in mouse derived muscle cell line, C2C12 cell, in response to uniaxial stretch stimulation. Therefore, this study aimed to investigate the function of APPL1 in C2C12 myotubes on GLUT4 translocation mechanism stimulated by mechanical stretch.

Materials and methods: C2C12 myoblasts were grown on an elastic silicone chamber and promoted differentiation. Differentiated C2C12 myotubes were stimulated by cyclic uniaxial stretch (10% of initial length, 10 cycle/min) for 5 hours. Plasmid DNA was transfected into C2C12 myoblasts by electroporation. Glucose uptake was measured by enzymatic assay and the localization of APPL1 was examined by immunofluorescence. Compound C, an AMPK inhibitor, or protein kinase C (PKC) zeta pseudosubstrate, a PKC inhibitor, was administrated to block the activity of AMPK or PKC zeta pathway during stretch.

Results: Stretch increased glucose uptake, Akt, AMPK, and PKC zeta phosphorylation without affecting protein expression of these proteins and GLUT4. APPL1 localized at perinuclear in basal condition and moved to plasma membrane after stretch stimulation. Stretch-induced glucose uptake, AMPK, and PKC zeta phosphorylation were statistically increased by 26%, 28%, and 30%, respectively, in APPL1 transfected cells compared to non-transfected. On the other hand, stretch-induced Akt activation was suppressed by 30% in APPL1-overexpressed cells. Although compound C didn't affect on glucose uptake, PKC zeta pseudosubstrate suppressed glucose uptake induced by APPL1 by 67% in stimulated condition.

Conclusion: These results suggest that APPL1 is regulated by stretch stimulation and promote stretch induced glucose uptake in C2C12 myotubes mainly through PKC zeta dependent signaling pathway, but not AMPK dependent pathway.

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Acetic acid enhances glucose uptake and blood flow rates in the skeletal muscle in humans with impaired glucose tolerance

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Background and aims: Previous studies support the antiglycaemic effect of vinegar. However, the effect of vinegar on endothelial function and muscle glucose metabolism has not been studied in humans. This cross-over study aims to investigate the effects of vinegar on plasma glucose, insulin and lipid levels, as well as blood flow and muscle glucose uptake in subjects with impaired glucose tolerance (IGT), using the arteriovenous difference technique.

Materials and methods: Eight subjects with IGT (age 46±4years, BMI 29±2), without any medication therapy, were randomised to consume vinegar (30ml vinegar containing 6% acetic acid, 20ml water) or placebo (50ml water) before a mixed meal (566kcal; 75g carbohydrates, 26g protein, 6g fat). Plasma samples were taken at 15-60min intervals for 300min from the radial artery and from a forearm vein for measurements of glucose, insulin, triglycerides, non-esterified fatty acids (NEFA) and glycerol. Muscle blood flow was measured with strain-gauge plethysmography. Glucose flux was calculated as the

arteriovenous difference of glucose multiplied by the blood flow rates. The cross-over study was conducted 1 week later.

Results: Vinegar compared to placebo: 1) decreased arterial plasma insulin (8101 ± 1410 vs 12008 ± 1645 mU/L*min, $p=0.043$) 2) increased forearm blood flow (404 ± 97 vs 134 ± 71 ml/min/100ml tissue*min, $p=0.048$) 3) increased muscle glucose uptake (933 ± 74 vs 603 ± 108 μ mol/100ml tissue, $p=0.02$) 4) decreased arterial plasma triglycerides (45 ± 11 vs 86 ± 10 nmol/L*min, $p=0.036$), without changing NEFA and glycerol.

Conclusion: In individuals with impaired glucose tolerance, vinegar addition to a mixed meal results in an enhancement of muscle blood flow rates, an improvement of glucose uptake by the forearm muscle and a reduction of postprandial hyperinsulinaemia and hypertriglyceridaemia. From this point of view vinegar may be considered beneficial for improving insulin resistance and metabolic abnormalities in the atherogenic prediabetic state.

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Disclosing acute caffeine action on insulin sensitivity: effects on skeletal muscle

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Background and aims: Caffeine, a non-selective adenosine antagonist, is the behaviorally active substance most widely consumed in the world. When consumed regularly, this xanthine presents beneficial effects on type 2 diabetes and metabolic syndrome. However, the sensitizing effect of chronic caffeine intake contrasts with acute caffeine administration that has been associated with an increase in insulin resistance (IR). The aim of this work was to investigate the effect of acute caffeine administration on insulin sensitivity and the involvement of adenosine receptors. Additionally, the mechanism behind caffeine-mediated effects in skeletal muscle was assessed.

Materials and methods: In vivo experiments were performed in Wistar rats of both sexes, aged 3 months (200–350g) anesthetized with pentobarbitone (60mg/Kg). The effect of the acute administration of caffeine (0.001–5 μ M), DPCPX (A1 antagonist, 0.0005–5 μ M), SCH58261 (A2A antagonist, 0.0005–5 μ M) and MRS1754 (A2B antagonist, 0.001–5 μ M) on insulin sensitivity was evaluated by means of an insulin tolerance test. Skeletal muscle Glut4 and AMPK α 1 expression were quantified by Western-blot. The effect of A1 and A2B adenosine agonists on glucose uptake was evaluated. Sodium nitroprussiate (SNP, 10nM), a nitric oxide (NO) donor was used to evaluate the effect of NO on adenosine antagonism induced-IR.

Results: Acute caffeine decreased insulin sensitivity in a concentration dependent manner ($E_{max}=55.54 \pm 5.37\%$, $IC_{50}=11.61$ nM), an effect that is mediated by A1 and A2B adenosine receptors. Additionally, in skeletal muscle, acute caffeine administration did not modify AMPK expression, however it significantly decreased Glut4 by 23.23% and 31.81% (0.05 and 0.5 μ M of caffeine, respectively). We found that A1, but not A2B agonists significantly increased glucose uptake to 2.11 ± 0.04 nmol.mg⁻¹ tissue in skeletal muscle when compared to control (1.69 ± 0.04 nmol.mg⁻¹ tissue). SNP partially reversed DPCPX and MRS1754 induced-IR by 77.4 and 51.1%, respectively, when compared with the application of adenosine antagonists alone (KITT DPCPX= 2.12 ± 0.44 glucose.min⁻¹; KITT MRS1754= 2.16 ± 0.08 glucose.min⁻¹).

Conclusion: Acute caffeine administration decreases insulin sensitivity in a concentration dependent manner being this effect mediated by A1 and A2B adenosine receptors. In skeletal muscle, the effect of caffeine on insulin sensitivity involves a decrease in Glut4 expression and in insulin-dependent glucose uptake that is mediated mainly by A1 adenosine receptors. Additionally, adenosine-mediated insulin sensitivity seems to involve NO production and its sensitizing actions.

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Glucose uptake independent of insulin action: role of S-nitrosylated insulin chains

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Background and aims: Portal insulin is removed (50–70%) during first pass transit in the liver by a process called insulin clearance. Protein disulfide isomerase (PDI) mediates insulin clearance by reduction of insulin disulfide bonds, breaking it into A and B chains by a mechanism that requires glutathione (GSH). Previously, we reported that A/B chains of insulin could be S-nitrosylated (A-SNO and B-SNO) in vitro, forming nitrosothiols. After a meal, the increase of GSH levels together with the activation of parasympathetic nervous system which leads to nitric oxide (NO) release results in an increase in peripheral insulin sensitivity. A concurrent increase in hepatic PDI activity cleaves and nitrosylates A and B chain of insulin (A-SNO e B-SNO). Our hypothesis is that S-nitrosylated modified derivatives of insulin, A-SNO and B-SNO, produced during the insulin clearance process stimulates glucose uptake in skeletal muscle by a mechanism independent of the insulin signalling pathway.

Materials and methods: The presence of BSNO in the liver of Wistar rats was analyzed by immunoprecipitation of S-nitrosylation proteins following by western blot for B-chain. Physiological effects of S-nitrosylated modified derivatives of insulin were evaluated in differentiated skeletal muscle cells (L6-mycGLUT4 cells) and adipocytes (3T3-L1 cells). The cells were stimulated with 100nM insulin, A-chain, B-chain, A-SNO and B-SNO to evaluate glucose uptake using 3H-Glucose method. To analyse detailed signalling pathways activated by these insulin derivatives we performed cellular extracts and by western blotting technique we investigate the enrolled proteins.

Results: We detected the existence of BSNO in liver homogenates of rats and that BSNO levels were decreased (23% comparing with sham rats) in animals that were subjected to an hepatic denervation of parasympathetic nerves - NO pathway. We observed that A-SNO and B-SNO significantly stimulates glucose uptake in L6 muscle cells ($159.8 \pm 19.2\%$ and $204.8 \pm 30.2\%$ to control, respectively), similar to insulin stimulus (203.1 ± 15.0). The stimulation of glucose uptake was mediated by the activation of AMPK pathway, detected by the increase in phosphorylation of Thr172 AMPK. We also observed that these insulin derivatives did not activate the canonical insulin pathway. In adipocytes we observed that A-chain and B-chain insulin derivatives increase glucose uptake ($309.2 \pm 83.2\%$ and $319.0 \pm 23.6\%$ to control, respectively). In a lesser extent B-SNO also stimulates glucose uptake ($193.6 \pm 13.8\%$ to control). In adipocytes these derivatives does not activate either the canonical insulin pathway or AMPK signaling pathway.

Conclusion: As a conclusion insulin clearance process can lead to the formation of A/B chain and/or the respective S-nitrosylated derivatives. In adipocytes, mainly A-chain and B-chain stimulates glucose uptake. On the other hand when insulin derivatives are S-nitrosylated (A-SNO and B-SNO) they act on skeletal muscle promoting glucose uptake, through activation of AMPK signaling pathway.

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Treatment with small, subthyrotoxic doses of thyroxine can improve peripheral cellular glucose transport and insulin sensitivity in patients with type 2 diabetes

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Background and aims: Variation of plasma thyroid hormones' levels influences insulin sensitivity and peripheral glucose disposal. Administration of high doses of thyroxine to healthy humans induces insulin resistance, whereas moderate doses increase peripheral glucose disposal. We investigated the effect of the administration of small doses of thyroxine to healthy humans

and patients with type 2 diabetes on the insulin sensitivity indices, in vitro glucose uptake and GLUT4 recruitment in the plasma membrane of peripheral monocytes.

Materials and methods: Eleven healthy, euthyroid subjects (CON) with a micronodular texture of the thyroid gland, aged 40.8 ± 2.7 yrs, BMI 26.6 ± 0.83 kg/m², T3 118.1 ± 5 ng/dl (1.818 ± 0.077 nmol/l), T4 7.1 ± 0.3 µg/dl (91.377 ± 3.861 nmol/l), TSH 1.235 ± 0.109 µU/ml, and eleven treatment-naïve subjects with type 2 diabetes (DM) [aged 42.9 ± 3.8 yrs, BMI 27.48 ± 1.39 kg/m², T3 119 ± 5.7 ng/dl (1.832 ± 0.087 nmol/l), T4 8.13 ± 0.46 µg/dl (104.8 ± 5.93 nmol/l), TSH 1.51 ± 0.14 µU/ml], were studied before and after administration of 50 µg of thyroxine once daily for 2 months. The study was approved by the hospital ethics committee, and subjects gave informed consent. A meal was given to the participants. Blood was drawn before the meal for the study of glucose uptake and GLUT4 abundance in peripheral monocytes and several blood samples were drawn for 300min from a forearm deep vein and the radial artery for measurements of glucose, insulin and HOMA-IR and Matsuda indices. The insulin's effect on GLUT4 translocation from cytoplasmic depots to plasma membrane was studied by stimulating monocytes with various concentrations of the hormone (0, 25, 50, 100 and 200 µU/ml). The increment of enrichment of plasma membrane to GLUT4 is represented as %increment from baseline (0 µU/l) to maximal concentration (200 µU/l). Plasma membrane GLUT4 abundance and glucose uptake (fluorescent analogue 6-NBDG) were studied in isolated monocytes by flow cytometry.

Results: The area under the curve of the 6-NBDG uptake was significantly higher in both CON (pre treatment 7392.7 ± 577.4 vs. post treatment 9383.8 ± 460 , $P < 0.05$) and DM (pre treatment 5597 ± 245 vs. post treatment 8916 ± 1009 , $P < 0.05$) after the administration of thyroxine. The abundance of plasma membrane GLUT4 increased after the treatment in both CON (pre treatment $29.6\% \pm 3.76$ vs. post treatment $41.93\% \pm 3.72$, $P < 0.05$) and DM group (pre treatment $10.96\% \pm 2.25$ vs. post treatment $34.44\% \pm 5.08$, $P < 0.005$). Glucose uptake and GLUT4 abundance in plasma membrane of monocytes improved, TSH levels reduced significantly post-treatment ($P < 0.0001$). Glucose, insulin levels and HbA1c reduced significantly in DM group. HOMA-IR and Matsuda indices improved post-treatment.

Conclusion: Administration of small, subthyrotoxic doses of thyroxine to euthyroid diabetic patients and healthy individuals, can improve peripheral cellular glucose uptake and insulin sensitivity. This could be of therapeutic importance in insulin-resistant subjects.

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Administration of small doses of thyroxine to euthyroid type-2 diabetic patients increased insulin-stimulated peripheral glucose uptake and improved glucose tolerance

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Background and aims: Variation of plasma thyroid hormones' levels influences insulin sensitivity and peripheral glucose disposal. Administration of high doses of thyroxine to healthy humans induces insulin resistance, whereas moderate doses increase peripheral glucose disposal. We investigated the effect of the administration of small doses of thyroxine to healthy humans and patients with type 2 diabetes on postprandial forearm muscle glucose uptake, and insulin sensitivity indices.

Materials and methods: A meal was given to eleven healthy euthyroid subjects (aged 40.8 ± 2.7 yrs, BMI 26.6 ± 0.83 kg/m², T3 118.1 ± 5 ng/dl (1.818 ± 0.077 nmol/l), T4 7.1 ± 0.3 µg/dl (91.377 ± 3.861 nmol/l), TSH 1.235 ± 0.109 µU/ml) and eleven treatment-naïve patients with type 2 diabetes (aged 42.9 ± 3.8 yrs, BMI 27.48 ± 1.39 kg/m², T3 119 ± 5.7 ng/dl (1.832 ± 0.087 nmol/l), T4 8.13 ± 0.46 µg/dl (104.8 ± 5.93 nmol/l), TSH 1.51 ± 0.14 µU/ml). Blood was drawn for 300min from a forearm deep vein and the radial artery for measurements of glucose, insulin, and glucose uptake. Forearm blood flow (BF) was measured with strain-gauge-plethysmography. Forearm Glucose-uptake, HOMA-IR, hepatic-insulin-sensitivity-index and ISI-Index (0-120) were calculated. After the first meal-tolerance-test, treatment with 50 µg of thyroxine once daily was initiated for a 2-month period. Then a second identical test was repeated.

Results: TSH levels reduced significantly post-treatment (DM: 1.51 ± 0.11 vs. 0.79 ± 0.11 µU/ml, $p < 0.0001$). Glucose, insulin levels and HbA1c reduced significantly in the diabetic group, (2867 ± 117 vs. 2315 ± 108 mMmin, $p < 0.0001$), (30426 ± 5041 vs. 18230 ± 3495 mUmin, $p < 0.0001$), ($8.35 \pm 0.18\%$

vs. $7.31 \pm 0.256\%$, $p < 0.0001$), respectively. Peak-baseline BF and Glucose-uptake (AUC0-300min) increased significantly (1.952 ± 0.3 vs. 3.54 ± 0.26 ml/min per 100cc tissue, $p = 0.0009$) and (556 ± 71 vs. 973 ± 81 µmol per 100cc tissue, $p = 0.0026$), respectively. All insulin-sensitivity indices improved post-treatment (ISI-HOMA: 0.207 ± 0.024 vs. 0.44 ± 0.027 , $p < 0.0001$), (Gutt-Index: 34.3 ± 2.43 vs. 54.7 ± 5.3 mg.L2/mmol/mU/min). The patients' weight was stable within the treatment period.

Conclusion: Administration of small, subthyrotoxic doses of thyroxine to euthyroid diabetic patients and healthy individuals, can improve peripheral glucose disposal and overall insulin sensitivity. The insulin sensitizing effect of thyroid hormone "boosting" within normal levels, improved glycaemia and insulin sensitivity indices. This could be of therapeutic importance in insulin-resistant subjects.

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Testosterone improves glucose homeostasis by increasing glucose uptake and glycolysis in human liver cells

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Background and aims: Men with low testosterone levels are at an increased risk of developing type-2 diabetes (T2D). Restoring testosterone to within the normal range through replacement therapy (TRT) improves insulin resistance and glycaemic control in hypogonadal men with T2D. The cellular mechanisms underlying these actions remain unknown but may be due, in part, to an effect on the liver as a major metabolic organ involved in glucose regulation. We have previously shown in testicular feminised mice, which have low testosterone levels and a non-functional androgen receptor (AR), that hepatic expression of glucose regulatory targets were significantly reduced and that TRT had a beneficial effect. Here we investigate the effect of testosterone on glucose homeostasis in human liver cells as a mechanism to improve glucose control and T2D.

Materials and methods: Glucose uptake was assessed using 2-NBDG, a fluorescent glucose analogue, in HepG2 cells treated with either testosterone (1-100nM) or vehicle control for 24h. Metformin was used as a positive control (5mM, 10mM). Flutamide was used to block AR actions. XF metabolic assays (Seahorse Bioscience) were performed to further assess cellular bioenergetics and mitochondrial function. Cells were analysed for mRNA and protein expression of targets of glucose regulation (Hexokinase 4, HK4; Phosphofructokinase, PFK; Mitogen-activated protein kinase kinase, MAP2K), by qPCR and western blotting.

Results: Glucose uptake was increased in testosterone treated cells at 10nM ($117 \pm 3.8\%$ of control, $P \leq 0.05$) and 100nM ($117 \pm 4.3\%$, $P \leq 0.05$) concentrations compared to control. This increase was similar to that of Metformin 5mM ($113 \pm 3.5\%$, $P \geq 0.9$) and 10mM (122 ± 4.8 , $P \geq 0.9$). Flutamide had no effect on testosterone action on glucose uptake. Extracellular acidification rate as an indicator of changes in the rate of glycolysis were marginally increased in testosterone treated cells. Similarly, oxygen consumption rate as a function of mitochondrial respiration was slightly but not significantly increased in testosterone treated cells. HK4 protein expression was increased in 10nM testosterone treated cells versus vehicle control (0.56 ± 0.04 vs. 0.24 ± 0.11 arbitrary densitometry units [ADU], $P = 0.08$), and significantly at 100nM concentrations (0.68 ± 0.25 vs. 0.24 ± 0.11 ADU, $P \leq 0.05$). No difference was observed between treated and untreated cells for PFK and LXR protein expression and for mRNA expression of all targets.

Conclusion: Testosterone increases glucose uptake in HepG2 cells as a mechanism to potentially improve hepatic glucose control. This action may be via increased hepatic glycolysis involving the upregulation of HK4 expression (a key regulatory enzyme in glycolysis) or increased mitochondrial respiration. This effect may also be, in part, AR-independent. Testosterone may improve T2D in men via actions on hepatic glucose regulation.

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Impaired hepatic insulin extraction in type 2 diabetes

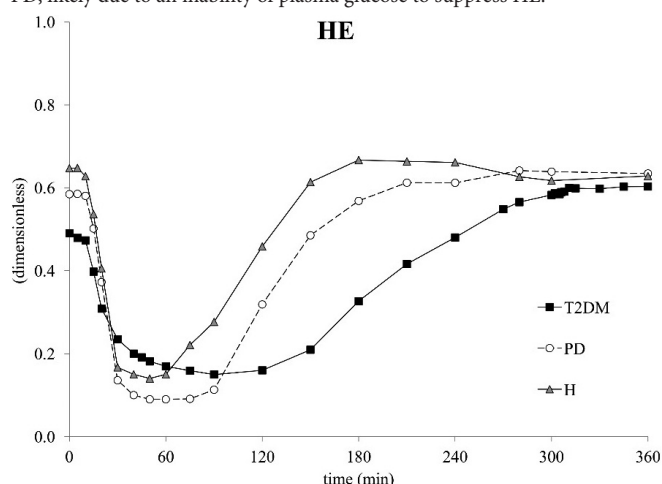
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Background and aims: The evaluation of hepatic insulin extraction (HE) during a meal is fundamental for understanding the regulation of carbohydrate metabolism in healthy (H), prediabetic (PD), and type 2 diabetic (T2DM) subjects. In this study we use a new physiologically-based mathematical model for estimating HE across the spectrum of glucose tolerance.

Materials and methods: To this purpose we studied 18 H (age=49.6±8.2 yr, BMI=27.7±3.1 kg/m²), 35 PD (both impaired glucose tolerant and impaired fasting glucose; age=53.2±7.7 yr, BMI=30.1±5.0 kg/m²) and 22 T2DM subjects (age=53.9±6.9 yr, BMI=32.7±4.9 kg/m²). Each subject underwent a standard mixed meal (75 g glucose, 43% carbohydrate, 17% protein, 40% fat). Frequent plasma sampling was undertaken in all the subjects in order to measure plasma glucose, insulin and C-peptide concentrations. The model assumes that C-peptide kinetics is described by a two-compartment model, insulin kinetics by a three-compartment model, and insulin secretion is a sum of two components, i.e. one proportional to glucose rate of change and one proportional, with a constant delay, to glucose concentration. HE suppression is assumed to be linearly dependent on plasma glucose concentrations. Basal (HE_b) and total (HE_{tot}) HE indices can be derived from the model parameters, as well as a new index quantifying HE sensitivity to glucose (S_G^{HE}).

Results: The model well fitted C-peptide and insulin data in all the subjects, also providing precise parameter estimates. Rank sum test showed that HE_b and HE_{tot} are significantly lower in T2DM than H subjects (HE_b=49% vs. 64%, p=0.01; HE_{tot}=43% vs. 59%, p=0.04). Moreover, S_G^{HE} is significantly lower in T2DM than PD (S_G^{HE}=0.10 vs. 0.14 l/mmol, p=0.006) and H subjects (S_G^{HE}=0.10 vs. 0.13 l/mmol, p=0.02). Figure 1 shows the average HE profiles reconstructed by the model in the three groups: evidently the basal state is impaired in T2DM, as it is confirmed by the statistical test, moreover, in T2DM, the profile has a longer low phase, and recovers slower than H and PD subjects.

Conclusion: The new physiological model for the quantification of HE, initially developed in H subjects, has been successfully used both in PD and T2DM. This model allows the estimation of a new index measuring HE sensitivity to glucose. This was significantly lower in T2DM compared to H and PD, likely due to an inability of plasma glucose to suppress HE.



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The effect of glycaemic variability on expression of genes involved in the development of hyperglycaemia induced tissue damage

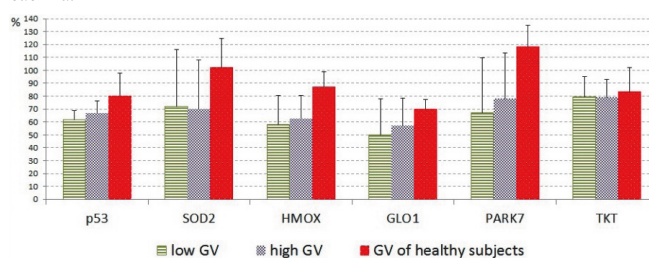
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Background and aims: Large prospective studies provided evidence of relationship between long-term diabetes compensation expressed as HbA1c and the risk of diabetic complications. However, HbA1c and diabetes duration explain only small portion of complications risk. It is well known that patients with the same HbA1c differ in amplitudes and duration of glycaemic excursions. Therefore, short-term (i.e. during the day) glycaemic excursions, so called glycaemic variability (GV), are debated currently. Animal experiments and *in vitro* studies showed that oscillating glucose may have more deleterious effect on cells due to higher production of superoxide than hyperglycaemia itself. Therefore, aim of our study was to evaluate the effect of GV on the expression of genes whose products are involved in the development of tissue damage in diabetes. We compared the effect of low and high GV of diabetics, GV of healthy subjects and chronic normo- and hyperglycaemia. Specifically, we detected gene expression of glyoxalase 1 (GLO1), heme oxygenase (HMOX), receptor for advanced glycation end-products (RAGE), p53, superoxide dismutase (SOD2), DJ-1 (an enzyme with glyoxalase activity), transketolase (TKT) and thiamine pyrophosphokinase (TPK1).

Materials and methods: Primary human umbilical vein endothelial cells (HUVEC) were cultured 24 hours in the following settings mimicking situation in humans (both diabetics and healthy) and in commonly used *in vitro* hyperglycaemia model: (A) high GV (standard deviation [SD] > 5), (B) low GV (SD < 3), (C) non-diabetics (SD = 1), (D) continuous normoglycaemia (5 mmol/l) and (E) continuous hyperglycaemia (25 mmol/l). Based on real 24 hours glycaemic curves from type 1 diabetics (3 patients with low GV and 3 with high GV) and healthy subjects (2 curves) we created 24 hours profiles with medium change after 2 hours. After 24 hours cells were harvested and RNA isolated and reverse transcribed using commercial kits (Roche). Gene expression was determined using quantitative PCR with predesigned probes (TaqMan™ Assay) with β-actin as a reference gene.

Results: HMOX expression was significantly lower in both low and high GV compared to GV of healthy subjects (P = 0.04, Mann-Whitney test). Similar pattern was also found in four of the selected genes (p53, SOD2, GLO1, DJ-1) although statistically not significant (all P > 0.05, Mann-Whitney test). Expression of all genes with exception of DJ-1 was significantly higher in continuous normoglycaemia when compared to low and high GV (all P < 0.04, Mann-Whitney). Finally, no significant difference was found between continuous hyperglycemia and low and high GV (all P > 0.05).

Conclusion: Results of our pilot study indicate that the effect of either low or high GV on expression of selected genes involved in diabetes induced tissue damage does not significantly differ from the effect of continuous hyperglycaemia.



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PS 035 Mechanisms of insulin action in non-human models

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Beneficial roll of TAZ modulator, TM-25659, in obese and diabetic conditions

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Background and aims: The transcriptional co-activator with PDZ-binding motif (TAZ) inhibits adipocyte development by intervention with peroxisome proliferator-activated receptor (PPAR)-gamma. Recently, PPAR-gamma has been implicated in muscle lipid accumulation. Therefore, we investigated the effects of TAZ modulator on skeletal muscle function in C2 myotubes and in C57BL/6J mice. In a previous study, Jang et al identified 2-butyl-5-methyl-6-(pyridine-3-yl)-3-[2'-(1H-tetrazole-5-yl)-biphenyl-4-ylmethyl]-3H-imidazo[4,5-b]pyridine (TM-25659) as a TAZ modulator and was shown to decrease weight gain in a high fat diet-induced obese model.

Materials and methods: We measured insulin signaling and glucose uptake using immunoblotting and 2-NBDG uptake. To elucidate the preventing mechanism of TM-25659 on palmitate-induced C2 myotubes insulin signaling, we performed real-time PCR with pro-inflammatory cytokines (TNF-alpha, IL-1-beta, IL-6 and MCP-1) mRNA. Male C57BL/6J mice that were fed a HFD for 8 weeks were randomly assigned for an additional 6 weeks to 3 groups: normal diet (ND), HFD, HFD+TM-25659. After treatment, oral glucose tolerance test (OGTT) and intraperitoneal insulin tolerance test (IP-ITT) were performed to evaluate anti-diabetic effects. After sacrifice, tissue extracts of the soleus muscle were obtained for quantification of insulin signaling through Akt phosphorylation proteins by immunoblotting.

Results: In our present study, we confirmed TM-25659 considerably inhibits palmitate-induced insulin resistance on C2 myotubes. Moreover, we showed that TM-25659 significantly suppresses high fat diet (HFD) induced insulin resistance in C57BL/6J mice skeletal muscle. Consequently, the TM-25659-treated group showed improved HFD-reduced glucose tolerance, increased insulin sensitivity, and increased AKT phosphorylation protein. Also, TM-25659 down-regulated expression of genes involved in pro-inflammation.

Conclusion: These results suggest TAZ modulator, TM-25659, play a beneficial role in controlling insulin resistance in obese and diabetic conditions.

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Human skeletal muscle clock: implications in myokine secretion in physiology and pathophysiology

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Background and aims: Circadian rhythms are present in many organisms of different complexity, representing an adaptation mechanism allowing adjustment of organism's internal processes to the external world. In mammals circadian system is organized in a hierarchical structure with a central pacemaker residing in hypothalamic suprachiasmatic nuclei that in its turn controls peripheral secondary clocks in organs including skeletal muscle. The aim of our study was to characterize molecular clock and its function in human primary skeletal muscle of healthy, overweight and obese subjects.

Materials and methods: Human primary myoblasts were derived from muscle biopsies of *Musculus gluteus maximus*. Bioluminescent reporters *Bmal1-luciferase* and *Per2-luciferase* were introduced by lentiviral transduction, and allowed visualization of the oscillatory profiles of *Bmal1* and *Per2* continuously in living cells. For clock ablation myotubes were transfected with siRNA-targeting *Clock*. Secretory profiles of myokine IL6 (ELISA measurements), assessed in parallel with bioluminescence profile recording, were compared in myotubes with functional and ablated clock.

Results: Our experiments revealed that fully differentiated *in vitro* synchronized myotubes exhibited circadian rhythm with a period length of about 24.6 hours. Oscillation period tended to be longer in case of overweight and obese subjects. Endogenous core clock transcripts as well as bioluminescent profiles showed sustained circadian expression pattern with pronounced amplitude. Around 90% of *Clock* knockdown significantly flattened oscillatory profile of *Bmal1-luciferase* reporter and amplitude of endogenous core clock transcripts. Our results revealed that the primary myotubes with intact clock secret IL6 in the circadian manner. Moreover, remarkable down regulation of IL6 secretion was observed upon clock ablation, meaning that the clock machinery might regulate IL6 secretion in human skeletal muscle.

Conclusion: Our data demonstrate that the human skeletal muscle exhibits pronounced circadian oscillations that impacts on the muscle IL6 secretory function. Moreover, changes in rhythmicity of circadian machinery in human skeletal muscle might be associated with metabolic disorders such as obesity.

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Role of the RNA binding protein Sam68 in leptin and insulin signalling in granulosa cells

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Background and aims: Obesity is a medical problem not only because it is associated with type 2 diabetes and cardiovascular risk, but also because in reproductive-age women obesity is associated with polycystic ovary syndrome (PCOS), insulin resistance and decreased fertility. The RNA-binding protein Sam68 is expressed in granulosa cells and the knockout female mice are subfertile, with ovulation problems. Since we have previously found that Sam68 may be recruited to insulin and leptin receptors, we planned to study the participation of Sam68 in signaling and action of insulin and leptin receptors in granulosa cells. In addition, we aimed to study the expression of Sam68 in granulosa cells in response to leptin and insulin *in vitro*.

Materials and methods: Signaling was studied by immunoprecipitation and immunoblot of the phosphorylated proteins. The expression of Sam68 is inhibited by antisense strategy. The expression level of Sam68, and leptin and insulin receptors are quantified by qPCR and immunoblot.

Results: We have found that Sam68 is tyrosine phosphorylated by insulin or leptin stimulation in granulosa cells, recruiting Sam68 to signaling complexes and decreasing its RNA binding capacity. In addition, both insulin and leptin increase the expression of Sam68 in granulosa cells. Finally, full expression of Sam68 is required for the activation of PI3K and MAPK signaling pathways by insulin or leptin in granulosa cells.

Conclusion: Sam68 is recruited to leptin and insulin receptor signaling, its expression is induced by both hormones, and Sam68 is necessary for the full activation of insulin and leptin receptor signal transduction in granulosa cells. Sam68 may be a new important element in the ovarian insulin resistance and the decreased fertility found in obese women.

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Insulin regulates glycogen synthesis in endometrial epithelial cells through an increase in glycogen synthase

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Background and aims: Endometrial gland secretion of glucose, lipids, and protein supports blastocyst nutrition prior to establishment of placental circulation. The endometrial glands fill with glycogen during the secretory phase in preparation for implantation, and regulation of glycogen synthesis has been attributed to progesterone. However, we hypothesized that insulin regulates glycogen synthesis in endometrial epithelial cells as it does in traditional insulin targets such as liver and muscle.

Materials and methods: We isolated endometrial epithelial and stromal cells from eight healthy women, and treated the cells separately with insulin, medroxyprogesterone (MPA), or vehicle. After 48hours, the cells were lysed,

and glycogen was isolated and quantified. Total and phosphorylated glycogen synthase (GS) and glycogen synthase kinase 3 α/β (GSK3 α/β) were evaluated by western blot after a 30min or 48hr hormone stimulation. Endometrial tissue from 45 women distributed across the menstrual cycle was assessed for insulin receptor β (IR β) by PCR and IHC.

Results: We found that IR β had higher expression during the secretory phase of the menstrual cycle, with an increase in expression in both epithelial and stromal cells between day 16 and 24 of the menstrual cycle. In vitro MPA increases IR β expression over 25-fold in primary stromal cells ($p<0.0001$) and to a lesser extent in epithelial cells. Insulin induces a 4.5-fold increase in glycogen content in primary epithelial cells ($p<0.01$), whereas MPA did not directly alter glycogen content relative to vehicle. In stromal cells, neither insulin nor MPA altered glycogen content. Insulin induced phosphorylation of GSK3 α/β acutely, and a 3.6-fold increase in GS protein by 48 hours ($p=0.005$). **Conclusion:** We demonstrate that progesterone regulates IR β expression during the secretory phase, facilitating insulin action. Insulin, not progesterone, directly regulates glycogen synthesis through an increase in GS as well as acute phosphorylation events. This mechanism is novel and distinct from insulin regulation of glycogen synthesis in the liver and muscle that is accomplished predominantly through acute phosphorylation events of GSK3 α/β and GS. A lasting increase in GS in response to insulin enables endometrial epithelial cells to synthesize glycogen continuously, independent of short term nutrient flux. This distinct regulation of glycogen synthesis is a unique adaptation to allow glycogen accumulation in endometrium. These findings suggest that successful implantation requires insulin, and may explain why women with insulin resistance have lower implantation rates.

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ECSCR negatively regulates insulin sensitivity via PTEN stabilisation in endothelial cells

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Background and aims: Endothelial insulin resistance impairs insulin delivery into skeletal muscle, and insulin-dependent glucose disposal is reduced in obesity patients with type 2 diabetes mellitus (T2DM). Endothelial cell surface expressed chemotaxis and apoptosis regulator (ECSCR) is a cell-surface protein selectively expressed on endothelial cells. Recent study revealed ECSCR is potentially therapeutic target for T2DM to enhance Akt/eNOS phosphorylation and improve insulin sensitivity in Ecsr-deficient mice. However, the underlying mechanism is still unknown. We investigated the mechanism how ECSCR negatively regulate insulin signaling using human umbilical vein endothelial cells (HUVECs) and mouse pancreatic endothelial cell line (MS1).

Materials and methods: HUVECs and MS1 were transfected with vector expressing FLAG-MYC-tagged ECSCR and the pCMV6 control vector for overexpression study. HUVECs were transfected with siRNA for ECSCR and scramble control sequence for knockdown study. Protein degradation activities of PTEN and PTP1B were evaluated on HUVECs with 10 μ g/mL of cycloheximide. mRNA and Proteins from cells were collected for RT-PCR and western blot analysis. All results are representative of at least three independent experiments.

Results: ECSCR overexpression on HUVECs and MS1 suppressed basal phosphorylation of Akt at Ser473. ECSCR knockdown on HUVECs increased phosphorylation of Akt at Ser473. ECSCR knockdown also significantly increased genes expression of PTEN and PTP1B, which are negatively modulating insulin signal on HUVECs. However, only PTEN protein amount in HUVECs was decreased. To identify the mechanism how ECSCR modulates insulin signaling, we evaluated the protein degradation activity of PTEN and PTP1B on HUVECs. ECSCR knockdown strongly enhanced the degradation of PTEN, but not PTP1B. Insulin stimulation accelerated PTEN degradation in HUVECs with ECSCR knockdown.

Conclusion: Taken together, our data proposed new mechanism that ECSCR selectively stabilize PTEN protein to prevent from protein degradation in HUVECs. ECSCR inhibition will be potentially new class of therapeutic target to improve endothelial insulin sensitivity in T2DM.

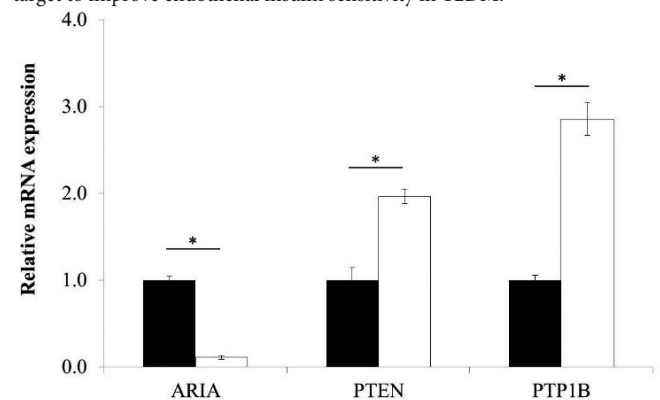


Fig 1. ARIA knock-down induces PTEN and PTP1B gene expression on HUVEC cells. Black bar represents Scramble group and white bar is ARIA knock-down group. Result represents mean \pm S.D. of relative gene expression ($n=3$). * $p<0.05$ (vs cells transfected with the scramble sequence, Student t-test).

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Insulin resistance of pancreatic alpha cells with age under high fat diet in mice

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Background and aims: Insulin and GLP-1 are strong inhibitors of glucagon release from pancreatic alpha-cells. Obesity causes insulin resistance of liver and skeletal muscle. However, it is not known if obesity causes insulin resistance in alpha-cells. In addition, the effectiveness of GLP-1 in ameliorating glucose tolerance and suppressing glucagon in aged organisms is not well understood. Therefore, we investigated insulin responsiveness of alpha-cells in a mouse model of obesity and GLP-1 (exendin-4) responsiveness in wildtype mice of different age

Materials and methods: Young (6 weeks) and old (8 months) C57BL6 wild-type mice ($n=27$) were fed with a high-fat-diet (HFD) (60% kcal of fat) over a period of 8 weeks to induce obesity and hyperinsulinaemia. Intraperitoneal glucose-tolerance-tests (ipGTT) were performed and levels of glucose, insulin and glucagon were assessed. Lean mice fed a standard diet served as controls ($n=27$). In addition, young (6 weeks) and old (8 months) C57BL6 wild-type mice were treated with exendin-4 (10nM/kg bodyweight; ip) over a period of 3 weeks followed by an ipGTT assessing glucose tolerance and glucagon secretion.

Results: Young as well as old mice gained approx. 40% of body weight after HFD compared to controls. In controls the blood glucose levels were 335 ± 66 mg/dl in young and 275 ± 72 mg/dl in old mice 30 min after glucose load with corresponding levels of insulin (360 ± 60 vs. 470 ± 120 ng/ml) and glucagon (26 ± 5 vs. 39 ± 11 pg/ml). In young mice on HFD the blood glucose levels were comparable with 335 ± 68 mg/dl after 30 min. In contrast, old mice after HFD showed significantly increased glucose levels of 503 ± 100 mg/dl at 30 min. Insulin levels increased 1,7fold (810 ± 286) after 30 min and 6fold (2.170 ± 280 ng/ml) after 120 minutes. Of note, glucagon levels were not suppressed (62 ± 2 pg/ml after 30min and 45 ± 4 pg/ml after 120min) despite the peaks in insulin release. In vitro experiments revealed that isolated islets from old animals after HFD showed no inhibition of glucagon release in response to insulin. Exendin-4 treatment improved glucose-tolerance in young and old mice at comparable levels (young: control 0 min blood glucose = 77 ± 31 , 30 min = 328 ± 41 ; exdn-4: 0 min = 100 ± 26 , 30 min = 163 ± 14 mg/dl; old mice: control 0 min = 82 ± 28 , 30 min = 314 ± 57 ; exdn-4 0 min = 95 ± 21 , 30 min = 160 ± 30 mg/dl). Interestingly, basal glucagon levels were higher in older mice than in young mice (56 ± 8 vs. 44 ± 6 pg/ml). The response in glucagon levels was comparable between controls and exendin-4 treated animals (young: control 0 min = 44 ± 6 , 30 min = 29 ± 9 ; exdn-4 0 min = 42 ± 12 , 30 min = 38 ± 14 pg/ml; old: control 0 min = 56 ± 8 , 30 min = 36 ± 9 ; exdn-4 0 min = 57 ± 10 , 30 min = 34 ± 5 pg/ml).

Conclusion: Higher age predisposes to impaired glucose tolerance after high-fat-diet in this animal model. Furthermore, high levels of endogenous

insulin in old mice on HFD fail to suppress glucagon release, indicating insulin resistance of glucagon producing α -cells. This was paralleled by *in vitro* findings with islets from old mice under HFD.

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Different proprotein convertases are involved in the maturation of the two insulin receptor isoforms

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Background and aims: The insulin receptor (IR) is expressed as two isoforms, IRA (exon 11-) and IRB (exon 11+), which originate from the alternative splicing of exon 11 and differ from 12 amino acids located at the carboxyl terminus of the extracellular α -subunit. The most significant difference between the two IR isoforms is that IRA is more responsive to activation by IGF2 than IRB. Thus, it is suggested that IRA and IRB are both responsible for metabolic effects (triggered by insulin) whereas, only IRA is responsible for the mitogenic/proliferate effects (triggered by IGF2). The two IR isoforms are matured in the Golgi apparatus by the proteolytic activity of the proprotein convertase furin. We analyzed if the absence or presence of exon 11 could affect the maturation of IR isoforms.

Materials and methods: IR isoforms were overexpressed in different cell lines. Proprotein convertases activity was modulated by pharmacological inhibition, specific knockdown and overexpression. IR maturation was analyzed by western blot.

Results: In furin-defective LoVo cells, IRB is more matured than IRA. In furin-expressing cells, both IR isoforms are equally matured and furin knockdown is more efficient in reducing the maturation of IRA than that of IRB. The global inhibition of proprotein convertase activity abolishes the furin-independent maturation of IRB, indicating the involvement of a proprotein convertase other than furin in this maturation. We demonstrate that in the absence of furin activity the proforms of IR are exposed at the cell surface where IRB (but not IRA) is matured by PCSK6. This difference in the maturation of IRA and IRB was used as an opportunity to modulate the balance metabolic/mitogenic signals emanating from IR. Furin inhibition reduces the signals triggered by IGF2 (emanating from IRA) without unduly affecting those triggered by insulin (emanating from IRB).

Conclusion: Our results are the first to reveal a difference in the maturation of IR isoforms. The maturation of IRA is almost strictly under the control of furin whereas that of IRB involves furin and PCSK6. Furin inhibition reduces the amount of mature IRA without drastically altering that of IRB, which reduces the signals triggered by IGF2 but not those triggered by insulin.

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FKBP5 gene polymorphisms and expression in human adipose tissue are associated with insulin resistance and type 2 diabetes

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Background and aims: Central obesity is associated with a cluster of metabolic alterations, which include insulin resistance (IR), dyslipidemia and cardiovascular disease. Glucocorticoid excess is associated with redistribution of fat from peripheral to central depots and with IR and development of diabetes. To identify potential novel mechanisms for IR we investigated dexamethasone-induced changes of gene expression in human adipose tissue.

Materials and methods: Subcutaneous and omental adipose tissue, obtained from 25 non-diabetic subjects (28–60 yrs; 20.7–30.6 kg/m²), was incubated without or with dexamethasone (0.003–3 μ M) for 24 h. Gene expression was assessed by microarray and real time-PCR. Protein levels were assessed by immunoblotting.

Results: FKBP5 (FK506 binding protein 5) was one of the genes responding most to dexamethasone. Gene expression increased up to 7-fold in a dose-dependent manner in both subcutaneous and omental fat depots ($p < 0.001$). The protein coded by this gene, the FKBP51, is an immunophilin, which means that it plays a role in the immune system. FKBP5 mRNA is widely expressed

in metabolically active tissues with the highest gene expression in muscle and adipose tissue. However, FKBP51 protein was about 10-fold higher in the omental than in the subcutaneous fat depot ($p < 0.05$), whereas the mRNA levels were similar. 80–90% of the FKBP5 protein in adipose tissue could be attributed to the adipocytes, while the stromal vascular cells contribute to 10–20% ($p < 0.05$). FKBP5 gene expression in the subcutaneous fat depot was positively correlated with markers of IR including, HOMA-IR and subcutaneous adipocyte diameter ($r = 0.59$, $p < 0.001$; $r = 0.48$, $p < 0.05$, respectively). In addition, FKBP5 gene expression in omental adipose tissue was associated with reduced insulin effects on glucose uptake in subcutaneous and omental adipocytes ($p < 0.05$). Interestingly, FKBP5 SNPs (e.g. rs2817056, rs2395635, rs1334894) were found to be significantly associated with type 2 diabetes and with 2-h OGTT glucose, HDL-cholesterol and triglycerides in publicly available datasets from large, population-based cohorts.

Conclusion: The FKBP5 gene is regulated by glucocorticoids in both subcutaneous and omental adipose tissue, and its expression in human adipose tissue is correlated to markers of IR. In addition, SNPs in the FKBP5 region are associated with type 2 diabetes and diabetes-related traits. We hypothesize that FKBP5 is a mechanism linking alterations in nutrient metabolism with immune function, both following glucocorticoid excess and in other conditions with IR. Further studies will address whether FKBP5 is causally linked to IR and whether the related mechanisms can provide novel pharmacological targets for the treatment of IR.

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Local overexpression of urocortin 3 in skeletal muscle of rats activates AMPK and Akt pathways and enhances glucose disposal

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Background and aims: Insulin resistance (IR) in skeletal muscle is an important component of both type 2 diabetes (T2D) and the syndrome of sarcopaenic obesity, for which there are no effective therapies. Urocortins (Ucn), ligands for corticotropin releasing factor receptors, are well-established as neuropeptides but also have roles in metabolism in peripheral tissues. We showed recently that global overexpression (OE) of Ucn3 resulted in muscular hypertrophy and resistance to the adverse metabolic effects of a high fat diet. Here, we aimed to establish whether short-term local Ucn3 OE could improve glucose disposal and insulin sensitivity in skeletal muscle through a paracrine mechanism.

Materials and methods: Ucn3 was overexpressed in right *tibialis cranialis* and *extensor digitorum longus* muscles of rats by *in vivo* electrotransfer and the effects on muscle size, metabolism, gene expression and signalling were studied versus the contralateral control muscles (electroporated with empty vector) after one week.

Results: No increase in whole muscle mass was detected after one week, but Ucn3 OE muscles showed 19% larger muscle fibre diameter ($p = 0.030$), perhaps mediated through the associated increases in insulin-like growth factor-1 (IGF1) and IGF1 receptor mRNA and increased Ser256 phosphorylation of forkhead transcription factor (FOXO1). Glucose clearance into test muscles, measured using tritiated 2-deoxyglucose after an intraperitoneal glucose load, was increased by 23% ($p = 0.018$) per unit mass, implying an insulin sensitising effect of Ucn3 OE on glucose disposal additional to the effect of hypertrophy alone to enhance total glucose uptake. This effect was associated with increased total glucose transporter protein levels (GLUT1: 34% increase, $p = 0.026$; GLUT4: 48% increase, $p = 0.0009$) and activation of two key signalling pathways mediating glucose disposal, the phosphoinositol 3-kinase/Akt (insulin signalling) and AMP-activated protein kinase (AMPK) pathways. Phosphorylation of insulin receptor substrate-1 (Tyr609), Akt (Ser473; by 72%, $p = 0.005$), Akt substrate of 160kDa (Thr642), glycogen synthase kinase-3 β (Ser9), AMPK (Thr172; by 27%, $p = 0.024$) and its substrate acetyl coA carboxylase (Ser79) were all significantly increased by Ucn3 OE.

Conclusion: Ucn3 OE enhances glucose disposal and insulin sensitivity in muscle by an autocrine/paracrine mechanism that is separate from its prohypertrophic effects, implying that such a manipulation may have promise for treatment of IR syndromes including T2D and sarcopaenic obesity.

Supported by: Wellcome Trust

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Physical inactivity and high fat diet synergistically enhance the accumulation of intramyocellular diacylglycerol and induce insulin resistance in murine soleus muscleS. Kakehi¹, Y. Tamura², S.-I. Ikeda³, R. Kawamori³, H. Watada²;¹Sportology Center, ²Metabolism and Endocrinology,³Sportology Center, Juntendo University, Tokyo, Japan.

Background and aims: Intramyocellular lipids (IMCL), especially intramyocellular diacylglycerol (IMDG) has been reported as one of the causes of insulin resistance in skeletal muscle. It has been demonstrated that physical inactivity and high fat diet (HFD) increase IMCL levels and induce insulin resistance, respectively. However, the combined effect of these two factors on insulin resistance has not been clarified yet.

Materials and methods: To elucidate them, C57BL6J mice were randomly assigned to four groups: control group, 24h hind-limb cast immobilization (HCI) group, short-term (2wk) HFD group, and 24h HCI after 2wk HFD group. Then, we evaluated ex-vivo insulin-stimulated 2-deoxy glucose uptake (2DG-uptake), insulin signal and intracellular fat composition in skeletal muscle.

Results: Twenty four hours HCI significantly decreased insulin-stimulated 2DG-uptake by 40%, while 2wk HFD did not alter 2DG-uptake. On the other hand, 24h HCI after 2wk HFD dramatically decreased insulin-stimulated 2DG-uptake by 74%. In parallel with decreased insulin-stimulated 2DG-uptake, we observed decreased insulin stimulated serine phosphorylation of Akt and tyrosine phosphorylation of insulin receptor substrate (IRS)-1 after 24h HCI, which were more exacerbated in 24h HCI after 2wk HFD group, while only 2wk HFD did not change these phosphorylation states. Concerning, it has been hypothesized that IMDG accumulation and PKC activation impairs insulin signal transduction. Consistent with this hypothesis, we found that 24h HCI increased IMDG in soleus by 192%, while the amount of intramyocellular triacylglycerol (IMTG) was not changed. In addition, whereas IMDG and IMTG were not changed by 2wk HFD feeding, 24h HCI after 2wk HFD dramatically increased IMDG and IMTG by 330% and 144%, respectively. In parallel with IMDG accumulation, we observed increased PKC ϵ activity in 24h HCI group and 24h HCI after HFD group by 151% and 210%, respectively. Associated with the IMDG accumulation, expression level of diacylglycerol acyltransferase (DGAT)-1, which converts diacylglycerol to triacylglycerol, was decreased in both HCI group and HCI after 2wks HFD group. In addition, activity of Lipin1, which converts phosphatidic acid to diacylglycerol, was increased in 24h HCI group and 24h HCI after HFD group by 141% and 210%, respectively

Conclusion: These results suggested that physical inactivity and HFD synergistically induce IMDG accumulation and insulin resistance in soleus muscle. Increased Lipin1 activity and decreased DGAT-1 expression might be involved in the mechanism of IMDG accumulation after physical inactivity and HFD.

PS 036 Mechanisms of insulin resistance in vivo

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Impact of hepatic lipid accumulation and composition on glucose tolerance and insulin sensitivity: a longitudinal study in male and female miceA.F. Soares¹, H. Lei², R. Gruetter^{1,2};¹Laboratory of Functional and Metabolic Imaging, ²Biomedical Imaging Research Center, École Polytechnique Fédérale de Lausanne, Switzerland.

Background and aims: Metabolic disruptions characterized by high hepatic lipid content (HLC) are associated with impairments in whole body glucose homeostasis. To gain insight on the role of hepatic lipids in the metabolic performance in the absence artificial metabolic stresses we measured non-invasively and longitudinally the HLC and profile in mice during adult development by Magnetic Resonance (MR) Spectroscopy *in vivo*. In parallel, mice were challenged with insulin and glucose tolerance tests.

Materials and methods: Male (N=10) and female (N=10) C57BL/6J mice were studied at 3 (3Mo), 7 (7Mo) and 10 months (10Mo) of age. Mice were scanned in a 14.1 T magnet with a ¹H quadrature surface coil over the abdomen. Localized ¹H spectra were acquired from a 8 μ l volume with stimulated echo acquisition mode sequence and the HLC expressed as the percent of total ¹H MR signal, with corrections for spin-spin relaxation effects. Additional spectra were acquired from the same volume with suppression of the water signal to enable the detection and quantification of all the lipid protons. The lipid profile was characterized by the following indices: saturated component (SC); unsaturated fatty acyl chains (UFA); mean number of double bonds per fatty acyl chain (ndb/FA), mean number of poly-unsaturated double bonds per fatty acyl chain (PUdb/FA) and per UFA (PUdb/UFA); mean chain length (MCL). OGTTs (1.5 g/Kg) and i.p. insulin tolerance tests (ITTs) were performed after a 6h-fast. Plasma insulin was determined by ELISA and insulin sensitivity estimated with the quantitative insulin check index (QUICKI) as the inverse of the log₁₀ sum of fasting insulin (μ U/ml) and fasting glucose (mg/dl). Data are expressed as mean \pm SEM. Statistical significance was accepted for a $P < 0.05$ (one-way ANOVA with Newman-Keuls post test) and correlations assessed by the Pearson r coefficient.

Results: In males, the HLC at 3Mo was $1.35 \pm 0.15\%$, increasing to $3.06 \pm 0.38\%$ at 7Mo, not different from $2.70 \pm 0.31\%$ at 10Mo. Females had higher HLC at 3Mo ($2.63 \pm 0.19\%$) but no further changes henceforward ($2.31 \pm 0.20\%$ at 7Mo; $2.36 \pm 0.20\%$ at 10 Mo). In males, the SC and MCL of hepatic lipids increased with age, with a trend for decreased PUdb/FA and PUdb/UFA with no changes in ndb/FA or UFA content. Females showed the same trends. Glycemia 3h-post ITT and 2h-post OGTT was lower in females, while QUICKI was higher. These scores were preserved until 10Mo in females. In males, glycemia 2-h post OGTT increased with age and the area above the curve (AAC) for the ITT decreased. In males, but not females, higher body weight correlated with hepatic lipid accumulation ($r = 0.7$); worse ITT scores correlated with higher body weight ($r = -0.6$) and HLC ($r = -0.7$) and lower PUdb/UFA ($r = 0.5$); worse OGTT scores correlated with higher HLC ($r = 0.4$).

Conclusion: In male mice, loss of insulin sensitivity correlated with weight gain, HL accumulation and lower poly-unsaturation. Glucose intolerance was specifically associated with HLC, suggesting a deleterious effect of lipids on the adaptation of hepatic metabolism to the fed state. This behaviour was not observed in females even if they showed similar HLC. In fact, the poly-unsaturation of HL in females didn't change with HLC, suggesting a positive effect of PUFA on preserving the hepatic metabolic performance.

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Liver specific G0/G1 switch gene 2 (G0S2) overexpression exacerbates hepatic insulin resistance by exacerbating hepatic steatosis but ameliorates hepatic fibrosis

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Background and aims: Hepatic steatosis is strongly associated with insulin resistance. Recently it has been reported that G0/G1 switch gene 2 (G0S2) inhibited the lipolysis activity of adipose triglyceride lipase. Moreover, we

confirmed that G0S2 protein content was increased in the livers of high fat diet (HFD) fed rats. However, the precise physiological role of hepatic G0S2 is still unknown. In the current studies, we investigated the effect of hepatic G0S2 on insulin sensitivity in HFD-fed male Wistar rats by overexpressing G0S2 proteins using an adenovirus (Ad) encoding mouse G0S2.

Materials and methods: Male Wistar rats were fed with 60% HFD for a total of 4 weeks. After 3-week feeding, the rats were injected with control Ad-GFP or Ad-G0S2. On day 7 post injection, glucose tolerance test and euglycemic-hyperinsulinemic clamp studies were performed after an 8-hour fast.

Results: There were no significant changes in the body weight and fasting Glucose levels between Ad-GFP and Ad-G0S2 rats. However, after the glucose load, the glucose levels were significantly higher in the Ad-G0S2 rats at 15 and 30 min. During the clamp studies, the glucose infusion rate required to maintain euglycemia was significantly decreased by 16% in Ad-G0S2 rats. Clamp hepatic glucose output was significantly increased by 31% in Ad-G0S2 rats, but there was no significant changes in insulin-stimulated glucose disposal rate between two groups. Furthermore, the Oil Red O staining revealed that overexpression of G0S2 significantly increased lipid accumulation in the liver. However, Masson trichrome staining revealed that overexpression of G0S2 significantly ameliorated fibrosis in the liver. Consistent with histological data, expression of TGF- β and Smad2 were significantly decreased in the livers of Ad-G0S2 rats

Conclusion: Overexpression of hepatic G0S2 protein exacerbates hepatic insulin resistance by the exacerbation of hepatic steatosis but ameliorates hepatic fibrosis.

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Fasting and postprandial hepatic energy metabolism in insulin resistant states

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Background and aims: Alterations of hepatic energy metabolism are discussed as a mechanism contributing to hepatic steatosis and insulin resistance. However, it remains unclear whether meal ingestion differently affects hepatic energy metabolism in insulin sensitive and insulin resistant humans.

Materials and methods: Young lean insulin sensitive (body mass index; 23.2±0.5 kg/m²), age-matched obese insulin resistant nondiabetic (OBE: 34.3±0.5 kg/m²) and elderly type 2 diabetes patients (T2D: 32.0±0.8 kg/m²) were examined (n=10 per group). Hepatic phosphorous compounds and fat content were quantified *in vivo* with ³¹P/¹H magnetic resonance spectroscopy before, 160 min and 240 min after ingestion of a high caloric mixed meal (652 kcal, 55% CHO, 15% protein, 30% fat). Whole body insulin sensitivity (M-value) was assessed with the hyperinsulinemic-euglycemic clamp and skeletal muscle oxidative capacity was assessed *ex vivo* with high-resolution respirometry applied in biopsy samples.

Results: OBE and T2D patients were similarly insulin resistant (M-value; OBE: 3.6±0.4 mg.kg⁻¹.min⁻¹; T2D: 2.5±0.7 mg.kg⁻¹.min⁻¹). OBE and T2D had 14fold (p=0.01) and 20fold (p=0.002) higher hepatic fat contents than lean persons. Fasting hepatic inorganic phosphate (Pi) was 42% lower in OBE (p=0.002) and 32% lower T2D (p=0.02) than in lean persons and correlated positively with M-value (r=0.63, p=0.001). After meal ingestion, hepatic ATP and Pi increased by 27% (p=0.02) and 33% (p=0.05) only in OBE. Circulating lipid peroxides transiently increased by 24% (p<0.05) at 180 min after the meal only in T2D. In parallel, mitochondrial oxidative capacity was reduced (p=0.01) along with augmented lipid storage (p=0.02) in skeletal muscle of T2D.

Conclusion: Obese insulin resistant persons respond to meal ingestion with augmented hepatic energy metabolism, whereas diabetes patients exhibit an oxidative stress response. These data indicate that obesity-associated adaptation of hepatic mitochondrial function is absent with the onset of type 2 diabetes.

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Serum hepassocin contributes to insulin resistance in type 2 diabetes C.-J. Chang¹, H.-T. Wu¹, H.-Y. Ou², H.-C. Hung², Y.-C. Yang¹, J.-S. Wu¹;

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Background and aims: Increased serum hepassocin concentrations were observed in patients with non-alcoholic fatty liver disease and diabetes. Although the mechanisms that hepassocin induces hepatic steatosis were clarified, the role that hepassocin plays in the development of insulin resistance and diabetes is still obscure.

Materials and methods: A total of 100 age- and sex-matched normal glucose tolerance (NGT), and newly diagnosed diabetic (NDD) patients were included (n=50 in each group) for the measurement of serum hepassocin concentrations by enzyme-linked immunosorbent assay. Insulin sensitivities in animals were determined using glucose tolerance test, insulin tolerance test, and euglycemic hyperinsulinemic clamp, as well as insulin signaling. Manipulations of hepassocin expression were achieved by portal vein injection of lenti-viral vectors containing hepassocin or short hairpin ribonucleic acid targeted to hepassocin.

Results: We found that the serum concentrations of hepassocin were significantly increased in patients with NDD (p<0.001) as compared with NGT subjects. Fasting plasma glucose (p<0.001), NDD vs. NGT (p<0.001) were significantly associated factors with serum hepassocin concentrations. Overexpression of hepatic hepassocin, as well as injection of hepassocin recombinant protein in mice increased fasting glucose levels, impaired glucose utility and induced insulin resistance. In addition, knockdown of hepatic hepassocin ameliorated insulin resistance in high fat diet-induced diabetic mice.

Conclusion: Hepassocin plays a role in the development of insulin resistance, and might be a novel therapeutic target for the treatment of diabetes.

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The vicious circle of prostaglandin- and cytokine-dependent hepatic insulin resistance: a key role of prostaglandin E2

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Background and aims: Hepatic insulin resistance and the ensuing impairment of hepatic glucose metabolism, which is a major contributor to hyperglycemia in metabolic syndrome and type II diabetes, are elicited by a variety of factors that act in parallel. In obesity, impaired fatty acid handling results in an activation of PKC isoforms. In addition, a low-grade inflammation prevails. Both contribute to the development of insulin resistance. The impact of cytokines on the insulin signalling cascade is well established. By contrast, the role of small lipid mediators like prostaglandins, which are released from resident liver macrophages and infiltrating immune cells in the course of inflammation, is not well characterized. The current study addressed this question.

Materials and methods: Male C57BL/6 mice were fed a modified western diet containing 50% of calories from fat, 1.25% cholesterol and 0.5% sodium cholate as well as 15% fructose in drinking water versus chow and water for 4 or 8 weeks. The human macrophage cell line U937, primary mouse and rat macrophages were incubated with LPS or PGE2. HepG2 cells or primary rat hepatocytes were pre incubated either with PGE2, IL 6, OSM or with conditioned media of PGE2-stimulated macrophages and subsequently stimulated with insulin. Gene expression and activation of insulin- and cytokine signalling were quantified by Western blot and quantitative RT-PCR.

Results: Hepatic expression of key enzymes in prostaglandin E2 (PGE2) formation, namely cyclooxygenase 2 (COX2) and microsomal PGE2 synthase 1 (mPGES1), was induced 2-4-fold in mice fed a modified western diet compared to controls suggesting an enhanced capacity of local PGE2 formation in these mice. In rat hepatocytes PGE2 interrupted Akt-dependent insulin signalling. It enhanced hepatic lipid accumulation by inhibiting mitochondrial fatty acid oxidation and VLDL formation. PGE2 acted by a distinct mechanism from cytokines, namely a sustained ERK1/2 activation, and synergistically with the cytokines interleukin-6 (IL-6) and oncostatin M (OSM), which attenuated insulin signalling by inducing SOCS3. In rat, mouse and human macrophages the release of these cytokines was stimulated by PGE2. Cytokines and PGE2 induced the PGE2-generating enzymes COX2 and mPGES1. While PGE2 besides IL-6 and OSM enhanced IL-1 β formation, TNF α expression remained unchanged or reduced. Conditioned

medium of murine or human macrophages treated with low doses of PGE2 stimulated the phosphorylation of STAT3 and induced SOCS3 in rat and human hepatocytes, while insulin-dependent glucokinase induction in hepatocytes was attenuated. These effects were abolished after parallel treatment of macrophages with the COX2-inhibitor indomethacin or incubation with an OSM-neutralizing antibody. In summary, in addition to directly interrupting insulin signalling pathway in hepatocytes, PGE2 may further impair hepatic insulin sensitivity in an autocrine feed-forward vicious cycle by inducing cytokines like OSM and its own synthesis in macrophages.

Conclusion: The study provides first evidence that in addition to established mechanisms prostaglandins have an impact on the development of hepatic insulin resistance. The enzymes involved in the prostaglandin synthesis and the receptors to which they bind on the different liver cell types might therefore be potential new drug targets for the treatment of hepatic insulin resistance.

Supported by: DGE

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Mitochondrial respiration in skeletal muscle, liver and fat does not differ in morbidly obese patients with and without type 2 diabetes

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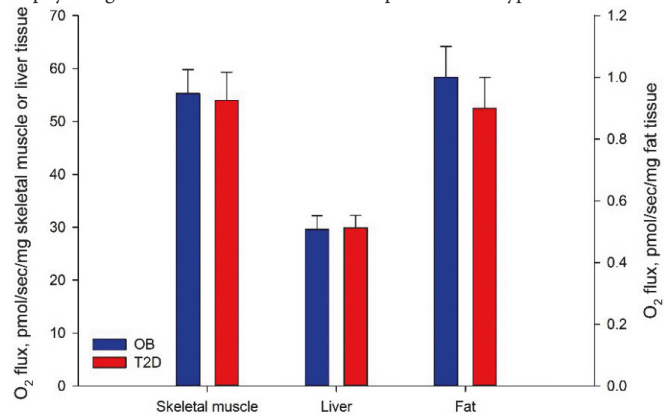
Background and aims: After an oral glucose load the uptake in skeletal muscle and liver accounts for ~80–90% of the ingested glucose. In patients with type 2 diabetes the ability to take up glucose in both tissues are severely impaired leading to decreased whole body glucose uptake and prolonged hyperglycemia. It has been speculated that the impaired glucose uptake partly is the result of an underlying mitochondrial dysfunction limiting the ability to oxidize glucose and fat. We have now compared the maximal coupled mitochondrial respiration in morbidly obese patients with and without type 2 diabetes by studying three different tissues (skeletal muscle, liver and fat) obtained from all patients.

Materials and methods: 16 morbidly obese patients (5M/11F; 7 with (T2D) and 9 (OB) without type 2 diabetes) reported to the lab twice 2 months prior to their scheduled Roux-En-Y gastric bypass operation. Body composition was assessed by a dual Energy X-ray Absorption scan and maximal oxygen uptake (VO₂max) was measured during a graded bicycle test. On a separate day skeletal muscle (m. vastus lateralis) and subcutaneous fat samples (abdomen) were acquired by a Bergstrom needle biopsy and subsequently a hyperinsulinaemic euglycemic clamp (80 mU/m²/min) was performed to measure whole body insulin mediated glucose uptake. Finally, a liver biopsy was obtained during the gastric bypass procedure. Prior to respiration analysis the skeletal muscle fibers were split and permeabilized in Saponin (50 µg/ml) for 20 min. Liver and fat tissue were dissected in 2 x 2 mm tissue samples before transferred to the respirometer. 2 µl digitonin (2.5 mg/ml) were initially added to the respirometer to permeabilize the fat tissue. Substrates were added consecutively: Malate (2 mM (liver: 6 mM)) and glutamate (10 mM); ADP (5 mM (liver: 1 mM)) and magnesium (20 mM (liver: 4 mM)); octanoyl carnitine (1.5 mM); succinate (10 mM).

Results: Weight (133±6 vs. 122±9 kg), BMI (43±2 vs. 41±1 kg/m²), age (36±3 vs. 40±6 years) and VO₂max (20±1 vs. 23±2 ml/min/kg) were similar in OB and T2D, respectively. Whole body glucose uptake tended to be higher in OB compared to T2D (4.8±0.5 vs. 3.6±0.3 mg/min/kg, respectively, P = 0.05). Maximal coupled respiration in skeletal muscle, liver and fat tissue are shown in Figure 1.

Conclusion: The main finding in the current study was the similar maximal coupled respiration in tissue samples from two different groups of patients with 25% different insulin sensitivity. Differences in body weight, age or aerobic fitness are not confounding factors. Thus, the mitochondria in patients with type 2 diabetes are able to consume oxygen at similar rates as healthy

obese patients and this speaks against an underlying mitochondrial dysfunction in insulin resistance. These data do not rule out that substrate sensitivity at physiological concentrations is altered in patients with type 2 diabetes.



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Failure of the skeletal muscle to contribute to diurnal glucose homeostasis in type 2 diabetes

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Background and aims: In health, food carbohydrate is stored as glycogen in skeletal muscle and liver, preventing a deleterious rise in osmotically active plasma glucose after eating. Glycogen concentrations increase sequentially after each meal to peak in the evening. Skeletal muscle accounts for the larger part of this diurnal buffering capacity. Approximately 30% of meal carbohydrate is stored in skeletal muscle compared with 20% in liver after a single meal. The effectiveness of this diurnal mechanism has not been previously studied in type 2 diabetes. We have quantified the changes in skeletal muscle and liver glycogen concentration before and after 3 meals consumed at 4 hours interval.

Materials and methods: We studied 40 (25M;15F) well controlled type 2 diabetes subjects on metformin only (HbA1c 6.4±0.07% or 47±0.8 mmol/mol) and 14 (8M;6F) glucose tolerant controls matched for age, weight and BMI. ¹³C Magnetic resonance spectroscopy at 3.0- Tesla, was used to quantify calf muscle and liver glycogen concentration in the fasting state (0830h) and at 2000h after 3 defined meals (2700kcal(M) and 2200kcal(F); 60%carbohydrate, 20%protein, 20%fat).

Results: Mean fasting plasma glucose and insulin concentrations were higher in the type 2 diabetes group (7.7±0.2 vs. 5.1±0.1mmol/L; P<0.0001) and (13.8±1.2 vs. 7.9±0.9mU/L; P=0.01) respectively. HOMA-IR was higher in the type 2 diabetes group (5.0±0.4 vs. 1.9±0.2; P<0.0001). Plasma glucose concentration measured 4 hours after the last meal (2000h) was higher in the type 2 diabetes group compared to the control group (6.5±0.2 vs. 5.7±0.2mmol/L; P=0.03). Plasma glucagon concentration rose similarly in the type 2 diabetes and control groups (70.4±4.7 to 108.5±7.5ng/L; P<0.0001 and 48.4±4.8 to 67.7±7.3ng/L; P=0.001). Muscle glycogen concentration increased by 17% in the control group (68.1±4.8 to 79.7±4.2mmol/L; P=0.006), and the change in diurnal muscle glycogen inversely correlated with HOMA-IR (r = -0.56; P=0.02). In contrast, there was no change in muscle glycogen in the type 2 diabetes group after day-long eating (68.3±2.6 to 67.1±2.0mmol/mol; P=0.62). Liver glycogen rose similarly in both normal control (325.9±25.0 vs. 388.1±30.3mmol/L; P=0.005) and type 2 diabetes groups (296.1±16.0 to 350.5±6.7mmol/L; P<0.0001).

Conclusion: In type 2 diabetes, the major physiological mechanism for skeletal muscle postprandial glycogen storage is completely inactive. This is directly related to insulin resistance, although liver glycogen storage is normal. Even in early well-controlled type 2 diabetes, failure of the buffering capacity of muscle glycogen stores is a major contributing factor to post prandial hyperglycemia.

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Skeletal muscle develops insulin resistance before subcutaneous adipose tissue. Study on obese post-menopausal women

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Background and aims: Obesity has deleterious effects on human health by enhancing the development of chronic diseases such as insulin resistance (IR) and type 2 diabetes (T2D). Nowadays, the hypothesis commonly admitted is that, during obesity, there is a hypertrophy and a hyperplasia of subcutaneous adipose tissue leading to its dysfunctions and increase lipolysis. Consequently to this adipose tissue dysfunctions, subcutaneous adipose tissue cannot expand more. Then free fatty acid (FFA) concentration increases in systemic circulation and lipids are ectopically stored in visceral adipose tissue, liver and skeletal muscle which affect importantly their function. Moreover, macrophages infiltration of adipose tissue induces a local chronic low-grade inflammation which develops to become systemic. This inflammation impairs the insulin response of insulin sensitive tissues (muscle, liver and fat) leading to peripheral IR. However, some obese people (25–30%) do not develop IR, and are metabolically healthy. In order to identify the early mechanisms leading from obesity to IR, we conducted a translationnal study both at systemic and tissue levels

Materials and methods: 31 post-menopausal women 50–65 years old, with no personal or familial history of type 2 diabetes were involved in this study: (i) 10 were lean (BMI 30 kg/m²) and insulin sensitive (HOMA-ir 3); OIR. Skeletal muscle and subcutaneous adipose tissue were obtained from biopsies respectively in the vastus lateralis and abdominal subcutaneous adipose tissue. We analyzed the insulin response, inflammatory state at systemic and tissues level, intramuscular fatty acid accumulation, angiogenesis and adipocytes size of subcutaneous adipose tissue. This protocol was approved by the local Ethic Committee (N°:2011.01.04, ANSM: B110204-20) and informed written consent was obtained from all participants.

Results: Ours results show a significant decrease in insulin sensitivity of OIR skeletal muscle compared to control (p=0.03) and OIS (p=0.04) skeletal muscle while subcutaneous adipose tissue remain insulin sensitive in all three groups of volunteers. Further, there is an increase of fatty acid accumulation in type I muscular fiber of OIR compare to control (p=0.02). Histological analysis show that macrophages infiltration is low in OIR's subcutaneous adipose and absent from OIS and CT volunteer's subcutaneous adipose tissue. Adipocytes size remains the same among three groups. However angiogenesis is significantly decreased in subcutaneous adipose tissue of OIR (p=0.02) and OIS (p=0.007) compared to control.

Conclusion: All these results suggest that skeletal muscle seem to be affected by IR before subcutaneous adipose tissue during IR development. The only one impairment which occurs in subcutaneous adipose tissue is the decrease in adipose tissue angiogenesis

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Serum myostatin and insulin resistance in postmenopausal women

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Background and aims: Myostatin is a secreted growth factor expressed in skeletal muscle, which negatively regulates skeletal muscle mass. Recent animal studies suggest an additional role for myostatin in glucose metabolism. We evaluated the possible associations between circulating levels of myostatin and glucose metabolism parameters in non obese women with postmenopausal osteoporosis.

Materials and methods: Prospective study including 28 women with postmenopausal osteoporosis, matched for age, BMI and bone mineral density (BMD), divided into two groups according to the antiosteoporotic drug: A) 60 mg Denosumab subcutaneous semiannually (n = 17) and B) 20 µg Teriparatide subcutaneous daily (n = 11), both for 3 months. The monitoring was conducted by the Bone Metabolism Unit of our hospital. We performed fasting plasma determinations of myostatin, glucose, peptide C, insulin, insulin resistance index (HOMA2-IR), insulin sensitivity (HOMA2-%S), insulin secretion (HOMA2-%β) and HbA1c at baseline and one week, one month and three months after treatment.

Results: Both groups were comparable at baseline in the analyzed parameters without significant differences between them. Serum myostatin levels remained unchanged regardless of treatment group. A global analysis of all determinations (108) shows a positive correlation between myostatin and peptide C (r = 0.231, p = 0.02), insulin (rs = 0.215, p = 0.02), HOMA2-IR (rs = 0.200, p = 0.04), HOMA2-%β (rs = 0.210, p = 0.03) and HbA1c (r = 0.209, p = 0.03) and an inverse correlation with HOMA2-%S (rs = -0.197, p = 0.04). No correlations were observed between myostatin and other related carbohydrate metabolism parameters as undercarboxylated osteocalcin, osteocalcin, IGF-1, vitamin D and BMI.

Conclusion: The findings of the present study show significant association between circulating myostatin and insulin resistance in non obese women with postmenopausal osteoporosis. This study supports the idea that myostatin may exert a negative effect on glucose metabolism acting as an independent mechanism in the pathogenesis of type 2 diabetes mellitus.

PS 037 Mechanisms of insulin action and inter-organ cross-talk

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Intracerebroventricular administration of vaspin triggers a brain-liver circuit to improve hepatic glucose homeostasis via the hepatic branch of the vagus

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Background and aims: Visceral adipose tissue-derived serpin (vaspin) is a member serpin A12 of the serine protease inhibitor family, which plays an important role in the modulation of glucose metabolism and insulin sensitivity. More and more findings raise the possibility that, vaspin regulates glucose metabolism and energy balance through its act on the central (hypothalamic) site. However, the regulatory role of vaspin in the brain in the control of liver glucose fluxes is unknown. In this study, we investigated the effects of vaspin signal conveyed by the hypothalamus on liver glucose fluxes in normal-chow-diet (NCD) or high fat diet (HFD)-fed male rats with or without hepatic branch vagotomy. We reported here a novel role of central vaspin in triggering a brain-liver molecular signaling pathway and neuronal network to control glucose production in vivo

Materials and methods: We established a model of central administration of vaspin. Hyperinsulinemic-euglycemic clamp and hepatic branch vagotomy were used to assess the effects of central vaspin on glucose metabolism and changes in liver and signaling pathway. [3-3H] glucose radioactivity was determined by scintillation counter. mRNA and protein expressions were measured by qRT-PCR and Western blot, respectively.

Results: We showed that central infusion of vaspin in HFD-fed animals significantly increased glucose uptake in peripheral tissues and decreased HGP. These changes were accompanied by a significant decrease in the hepatic glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) expression. In agreement with this, we also found that central vaspin in HFD rats activates the insulin receptor (InsR) → insulin receptor substrate-1 (IRS-1) → Akt kinase (Akt) → forkhead box-containing protein of the O subfamily 1 (FoxO1) signaling cascade in the liver leading to the increased insulin sensitivity and improved glucose metabolism.

Conclusion: Our findings suggest that hypothalamus is a site of vaspin action, where vaspin triggers a brain-liver circuit via the hepatic branch of the vagus nerve to inhibit glucose production and improve insulin resistance.

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Abnormal hepatic energy metabolism in type 1 diabetes associates with long-term metabolic control

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Background and aims: Insulin resistant patients with type 2 diabetes exhibit lower hepatic energy metabolism, which has been explained by increased lipid availability and/or impaired glycemic control. This study set out to examine the role of insulin resistance and glycemic control for hepatic energy metabolism in type 1 diabetes mellitus (T1D) patients with good glycemic control.

Materials and methods: Thirty-seven newly diagnosed T1D patients (age: 34±9 years; body mass index (BMI): 24.4±3.3 kg/m²) with known diabetes duration of less than one year underwent euglycemic-hyperinsulinemic clamps to assess whole-body (M-value) and hepatic insulin sensitivity (insulin-mediated percent suppression of endogenous glucose production). Hepatic fat content (HCL), γ-adenosine triphosphate (γATP) and inorganic phosphate (Pi) concentrations were quantified via magnetic resonance spectroscopy (³¹P/¹H-MRS) on a 3-T clinical MR scanner (Philips, Best, The Netherlands).

BMI- and age-matched healthy controls (CON; n=27, age: 38±9 years; BMI: 24.4±3.4 kg/m²) were also studied with ³¹P/¹H-MRS.

Results: Well-controlled T1D patients (HbA1c: 6.5±1.1%) presented high whole-body and moderate hepatic insulin sensitivity (7.8±2.9 mg*kg⁻¹*min⁻¹ and 62±24%). HCL was comparable between T1D and CON (1.7±3.1% and 1.9±3.9%). Hepatic γATP concentrations were 16% lower in T1D (2.22±0.54 vs CON: 2.61±0.55 mmol/l, P<0.01), whereas Pi levels were not different between groups. Within the T1D group, hepatic γATP related negatively with HbA1c (r=-0.42, P=0.01), whereas Pi related negatively to BMI (r=-0.39, P=0.02), waist circumference (r=-0.47, P=0.003) and HCL (r=-0.55, P<0.001). Neither hepatic γATP, nor Pi displayed correlations with whole-body and hepatic insulin sensitivity.

Conclusion: Hepatic γATP is reduced in patients with T1D even in the absence of liver steatosis, and associates with measures of long-term glycemic control, but not with hepatic and peripheral insulin sensitivity.

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Insulin resistance and beta cell function in nonalcoholic fatty liver disease (NAFLD)

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Background and aims: The global epidemic of non-alcoholic fatty liver disease (NAFLD) has led to the recognition of associated complications that include diabetes and cardiovascular disease in addition to the liver failure and/or hepatocellular carcinoma related to the nonalcoholic steatohepatitis (NASH). The increased risk of cardiovascular disease suggests that NAFLD is the hepatic component of the Metabolic Syndrome. The goal of this study is to evaluate β-cell function and insulin resistance in correlation to metabolic parameters in NAFLD/NASH patients to determine parameters to identify NAFLD patients at increased risk of diabetes.

Materials and methods: Charts were reviewed retrospectively from 200 consecutive patients in the Metabolic Liver Clinic. After excluding those with viral or autoimmune hepatitis, gluco-regulatory status was assessed by A1c and by OGTT with glucose & insulin measured at 0, 30, 60, 90 & 120 minutes plus c-peptide & proinsulin measured at 0 & 30 minutes. Homeostatic model assessment (HOMA) of β-cell function and insulin resistance (IR) were calculated. Subjects treated with insulin were excluded from this part of the analysis. We assessed correlations with fasting insulin, proinsulin, fasting c-peptide, insulin resistance as measured by HOMA-IR, pancreatic β-cell function, as measured by HOMA-β, BMI, lipids, liver enzymes and NASH FibroSURE®. Spearman correlation coefficient was used to measure the association between metabolic parameters.

Results: After excluding diabetes (whether known or newly diagnosed; n=64) 58 patients were found to have NAFLD and prediabetes. In the subjects with prediabetes the following significant correlations were found: HOMA-IR with BMI (p=0.022) but not A1c; fasting proinsulin with BMI (p=0.0081) and A1c (p=0.04), fasting glucose (p=0.006) with HDL (p=0.0007); fasting C-peptide with BMI (p<0.02), HDL (p=0.01) and HOMA-IR (p=0.0018); and FibroSURE® Steatosis Score with BMI (p<0.0001) and A1c (p=0.05). There was a significant negative correlation between HDL and fasting insulin level (p=0.0079). There was no correlation between c-peptide and FibroSURE® Steatosis Score. When evaluated by gender the majority of the significant correlations remained present in men but not in women with the exception of BMI (p<0.05 in women) and A1c (p<0.05 in women).

Conclusion: The majority of the patients referred to the Metabolic Liver Clinic for evaluation of NAFLD were found to have either Type 2 Diabetes or prediabetes, most of which was not previously diagnosed. Analysis of those with prediabetes found insulin resistance with elevated insulin, proinsulin and c-peptide levels suggesting that identification of the NAFLD had facilitated early diagnosis of prediabetes in these patients who still had significant beta-cell function. These data demonstrate that screening NAFLD patients is an opportunity to identify prediabetes at a stage when therapy has the potential to slow or prevent the progression to not only Type 2 Diabetes but also the cardiovascular disease that is significantly increased in this population. Studies are ongoing to identify medical strategies to improve the gluco-regulatory status of these NAFLD patients with prediabetes.

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The association of serum fibroblast growth factor 21 with insulin secretory function and metabolic parameters in type 2 diabetes

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Background and aims: Fibroblast growth factor 21 (FGF21) is a cytokine produced by the liver, adipose tissue, skeletal muscle, and the pancreas. Recently, FGF21 is suggested as a key metabolic regulator in glucose and lipid metabolism and its level in serum is higher in the patients with metabolic syndrome, type 2 diabetes and non-alcoholic steatohepatitis. On the other hand, diminished expression of FGF21 is shown in type 1 diabetes and latent autoimmune diabetes in adult (LADA). Type 2 diabetes is a heterogenic disease entity and patients with type 2 diabetes have diverse insulin secretory dysfunction. So, we investigated the association of serum FGF21 and insulin secretory function in patients who admitted our institute for poorly controlled blood glucose and needed insulin therapy.

Materials and methods: We recruited 154 patients (men 52; women 152) with type 2 diabetes who were not treat with insulin prior to informed consent from January 2011 to July 2013, who admitted Keimyung University Dongsan Medical Center. We reviewed personal history including drug history, anthropometric parameters, other metabolic parameters, and measured serum adiponectin and FGF21.

Results: Mean age was 55.4±14.0 in men and 61.4±14.1 in women and body mass index was 24.2±4.7 kg/m² in men and 25.0±4.2kg/m² in women. There was no significant difference in hemoglobin A1c (10.4% in men; 10.3% in women). Fasting C-peptide, insulin and homeostasis model assessment of insulin secretion (HOMA-IS) were used as assessment of insulin secretory function. Serum FGF21 level shows correlations with HOMA-IS and fasting insulin in men, but not in women. In addition, serum FGF21 level shows correlations with serum triglyceride, insulin and homeostasis model assessment of insulin resistance (HOMA-IR) in men, however, shows correlations with urine albumin/creatinine and HOMA-IR in women. As a result of multivariate linear regression analysis, only HOMA-IS has a significant correlation with serum FGF21 level. Serum adiponectin level shows negative correlations with duration of diabetes, BMI, total cholesterol and low density lipoprotein in men and shows negative correlation with BMI, waist circumference, total cholesterol and triglyceride, high density lipoprotein in women.

Conclusion: Consequently, this study exhibits a positive correlation of serum FGF21 level with an index of insulin secretory function in men with type 2 DM and supports the result of another study with type 1 diabetes and LADA.

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Novel paracetamol glucuronide derivative for quantifying gluconeogenesis using the deuterated water method

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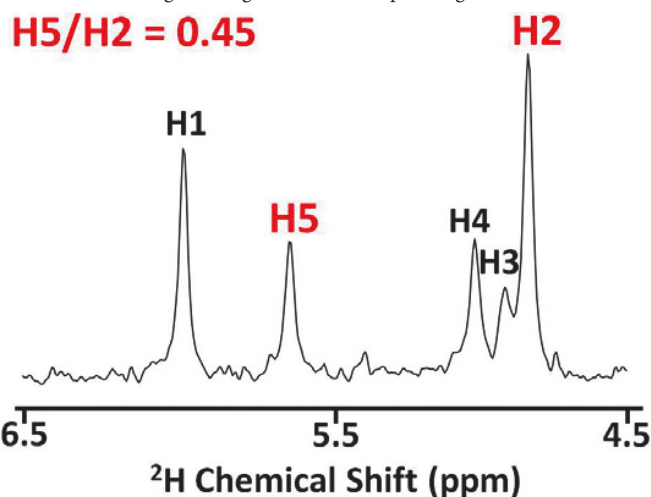
Background and aims: In fasted humans, the analysis of plasma glucose ²H-enrichment in position 5 relative to position 2 (H5/H2) following ingestion of deuterated water (²H₂O) is widely used to measure the fractional contribution of gluconeogenesis to endogenous glucose production (EGP). This information can be noninvasively obtained via urinary Paracetamol glucuronide (PG): a product that is more stable and abundant compared to blood glucose. However, current methods for derivatizing urinary PG to a form that can be analyzed for positional ²H-enrichment by NMR or MS are laborious and ill-suited for high-throughput studies. We developed a novel procedure where urinary PG is derivatized to 5-O-acetyl monoacetone glucuronic lactone (MAGLA). The chemical transformation and purification steps are robust and amenable to automation and parallel processing and yield resolved signals for H5/H2 analysis by ²H NMR (see Figure). We applied this procedure to a study where ²H₂O and Paracetamol were given to overnight fasted

healthy subjects and compared H5/H2 of plasma glucose with that of urinary glucuronide.

Materials and methods: Eleven subjects were admitted to the clinical research unit on the evening before the study and provided a standard supper at 17.30. Each ingested 0.5 g ²H₂O/kg body water in three equally divided doses at 20:00, 22:00, and 24:00. At 05:00 the following day, Paracetamol (0.5g) was given and a primed infusion of [6,6-²H₂]glucose was initiated. Urine was sampled from 06:00-08:00 and blood was sampled at 07:40. Plasma glucose and urinary PG enrichments of positions 5 and 2 were measured by ²H NMR following their derivatization to monoacetone glucose and MAGLA, respectively. The fractional gluconeogenic contributions to EGP were calculated from plasma glucose H5/H2. These were compared with estimates obtained from urinary glucuronide H5/H2.

Results: The overall yield of MAGLA from urinary glucuronide was 20–40% and the preparations gave well-resolved ²H NMR signals for quantifying H5/H2. Analysis of MAGLA yielded identical estimates of fractional gluconeogenic contributions to EGP to that of plasma glucose (54 ± 2% versus 55 ± 3%, respectively). Furthermore, a Bland-Altman analysis indicated agreement at the 95% confidence level between the sets of plasma glucose and urinary MAGLA measurements.

Conclusion: The conversion of urinary Paracetamol glucuronide to MAGLA is a relatively simple and robust procedure for obtaining estimates of gluconeogenesis using ²H₂O, or indeed any gluconeogenic tracer. For overnight-fasted healthy subjects, ²H NMR analysis of MAGLA provides identical estimates of fractional gluconeogenesis to that of plasma glucose.



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BTG2 regulates hepatic gluconeogenesis through Nur77 in diabetes

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Background and aims: Beta cell translocation gene 2 (BTG2) is a member of a family that is involved in cellular functions. Herein, we demonstrate that BTG2 regulates glucose homeostasis via up-regulation of Nur77 in diabetic mice.

Materials and methods: Diabetes was induced in wild-type (WT) and db/db mice by i.p injections of streptozotocin (80 mg/kg body weight). GTT and ITT were performed with db/db mice. Mice were infected with adenoviral vector expressing Btg2(1×10⁹ pfu) by tail vein injection. Gene expression and induction level were measured by qPCR and immunoblotting.

Results: Hepatic BTG2 gene expression was elevated by fasting and forskolin. Overexpression of BTG2 increased the expression of hepatic gluconeogenic genes, glucose output, and subsequently impaired glucose and insulin tolerance. Up-regulation of the transcriptional activity of Nur77 and gluconeogenic genes, and glucose production by forskolin were observed by BTG2 transduction, but not in BTG2 knockdown. BTG2-stimulated glucose production and G6pc promoter activity was attenuated by dominant-negative Nur77, but not in Pck1 promoter. Chromatin immunoprecipitation assays showed that BTG2 induced Nur77 occupancy on the glucose-6-phosphatase gene promoter via a physical interaction. BTG2 gene expression was increased in streptozotocin-treated and db/db mice. Finally, impairment of

glucose homeostasis such as the increase of blood glucose, glucose intolerance and insulin intolerance was elevated in diabetic mice, whereas this phenomenon was abolished in knockdown of BTG2.

Conclusion: These data suggest that BTG2 participates in the regulation of hepatic glucose homeostasis, which involves that BTG2 might serve as a potential therapeutic target for combating metabolic dysfunction.

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New diabetes drug targets from structural characterisation of liver glycogen

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Background and aims: Glycogen is a highly branched polymer of glucose, functioning as a blood-glucose buffer. It comprises relatively small beta particles, which may be joined as larger aggregate alpha particles. The molecular size distributions from size-exclusion chromatography of liver glycogen from non-diabetic and diabetic mice show that diabetic mice have impaired alpha particle formation in liver glycogen, with diabetic mice unable to form as many large glycogen particles as non-diabetic controls. There is also reason to believe that the degradation of larger particles back to glucose will be slower, and hence under better control, than smaller beta particles. Further analysis into the nature of “healthy” glycogen has provided evidence that these alpha particles are held together via an unknown glue that is most likely proteinaceous. This study probes the structure of glycogen alpha particles by analyzing their digestion during acid hydrolysis and by comparing the in vitro digestion rate of glycogen phosphorylase for glycogen of various sizes. This greater insight into the internal nature of alpha particles is an important step into finding potential drug targets that would allow diabetic sufferers to form these larger alpha particles and hence have better blood-sugar control.

Materials and methods: The digestion of glycogen by acid hydrolysis was investigated by analyzing the size distributions, obtained using size-exclusion chromatography, of pig-liver glycogen and phytoglycogen at various time points in a low-pH buffer. In addition, glycogen from the livers of wild-type mice were extracted and fractionated into different sizes using sucrose density centrifugation. The size distributions of these fractions were compared using size-exclusion chromatography. The in vitro kinetics of the initial rates of phosphorylase degradation were examined and compared for each fraction using an assay that utilized the production of NADPH in the reaction mixture, which has an absorbance at 340 nm.

Results: The acid hydrolysis experiments show that the alpha particles in the liver glycogen degrade via a different mechanism to that of the smaller beta particles, indicating a different type of chemical bonding (most likely proteinaceous). The in vitro kinetics analysis of glycogen phosphorylase on fractionated glycogen showed that larger glycogen particles indeed had a lower initial rate of phosphorylase degradation.

Conclusion: The difference in kinetics of the acid hydrolysis of alpha and beta particles indicates that they have a different mechanism of biosynthesis. The hypothesis that larger glycogen alpha particles are more slowly degraded by glycogen phosphorylase, presumably due to a lower surface area to volume ratio, was supported by the present data. This shows that diabetic mice, which have been shown to lack the larger alpha particles, may be vulnerable to having a fast, uncontrolled release of glucose. Because alpha particles have been shown here to have a separate mechanism of formation to smaller beta particles, the activation of this process in diabetic sufferers is now a potential drug target.

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Post-prandial and ChREBP mediated PANDER expression

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Background and aims: Pancreatic-Derived Factor (PANDER, FAM3B) is a novel endocrine pancreatic secreted protein mainly characterized from this tissue. PANDER regulates hepatic insulin sensitivity and overall glycaemic levels. However, recent research now suggests that PANDER is poten-

tially expressed within the liver as well. Our previous data have shown the PANDER promoter to be highly active and glucose-responsive in a murine liver-derived cell line. To further investigate the regulation and expression of hepatic derived PANDER, we examined hepatic PANDER levels in mice and characterized the minimal element of the PANDER promoter in a liver-derived cell line.

Materials and methods: Western analysis was performed to evaluate the presence of PANDER protein within murine tissues. Promoter deletion analysis and co-transfection experiments were performed using PANDER promoter-luciferase constructs and ChREBP expression plasmids in a murine liver-derived cell line, BNL-CL2.

Results: Initial western analysis performed on 15 different murine-derived tissues revealed robust PANDER expression within the murine liver and to a lesser extent in the brain and ovary. Furthermore, hepatic PANDER levels were measured in the fasted and fed state of our PANDER transgenic and control mice and revealed increased levels in the post-prandial state. The minimal element was identified between -293 to -3 bp of the hepatic transcriptional start site. The region between -193 to -93 bp of the TSS was demonstrated as being necessary for basal promoter activity. This region contains three E-box elements which may serve as a binding site for the critical hepatic transcription factor carbohydrate responsive element binding protein (ChREBP). Co-transfection reporter studies revealed that PANDER promoter activity is significantly upregulated ($p < 0.05$) in response to increased levels of ChREBP under both starved and elevated glucose conditions.

Conclusion: Our western analysis results confirm the presence of PANDER protein within murine liver tissue as well as the upregulation of PANDER protein in the fed state. This supports our previous finding that elevated glucose levels result in increased PANDER promoter activity. In addition, we identified a minimal element within the PANDER promoter which contains a putative binding site for the glucose-responsive transcription factor ChREBP. Our co-transfection experiments suggest that ChREBP may play a role in mediating the glucose-responsiveness of the PANDER promoter within the liver, and indicates another mechanism for regulation of hepatic insulin signaling via PANDER.

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Soluble RAGE is unlikely to induce type 2 diabetes by inducing insulin resistance or beta cell dysfunction

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Background and aims: Soluble receptor for advanced glycation end product (sRAGE) is considered as protective against diseases originating from RAGE activation. However, recent studies have suggested that sRAGE may be involved in the pathogenesis diabetes mellitus. A declining level of sRAGE has been suspected to be a predictor of type 1 diabetes, and an independent association has been found between low levels of sRAGE and development of type 2 diabetes. To further explore the involvement of sRAGE in the pathogenesis of type 2 diabetes, present study was designed to assess whether sRAGE level alters in prediabetic subjects and whether sRAGE level correlates with insulin resistance and beta cell function.

Materials and methods: A total of 128 subjects were recruited from those who came for diabetes screening at our medical university. Subjects were grouped as control (normoglycemic), pre-diabetic and diabetic based on their blood glucose (fasting and 2 hrs after 75 grams glucose load) and HbA1c levels following ADA criteria. Subjects suffering from complications of diabetes mellitus, hypertension, chronic liver and kidney diseases, and regular drug users were excluded. Fasting serum sRAGE levels were measured by ELISA (R&D Systems), and insulin levels were measured by enzyme immunoassay technique (Abbott AxSym System). Insulin resistance (HOMA2-IR) and beta cell function (HOMA2-%B) were calculated using HOMA2 calculator. To achieve a normal distribution some values were appropriately transformed before statistical analysis in SPSS 17.0 software. A p value of < 0.05 was considered statistically significant.

Results: Age (40.2 ± 8.7 years) and sex ($m=58$, $f=70$) distribution of the study subjects were not different among control ($n=30$), pre-diabetic ($n=42$) and diabetic ($n=56$) groups. Fasting glucose and HbA1c levels were found significantly ($p < 0.001$) elevated in diabetic group compared to control and pre-diabetic groups. Fasting insulin levels were found similar among control

(11.8 ± 6.8), pre-diabetic (14.2 ± 8.7) and diabetic (13.8 ± 11.0 $\mu\text{U/mL}$) groups. However, HOMA2-IR values [median (IQR)] were 1.37 (0.99–2.12) for control, 1.71 (1.27–2.60) for pre-diabetic and 1.95 (1.13–3.23) for diabetic groups ($p < 0.05$); and HOMA2-%B values were 140% (99–198) for control, 132% (107–167) for pre-diabetic and 46% (24–89) for diabetic groups ($p < 0.001$). However, serum sRAGE levels did not show any difference among control [626 (413–826) pg/mL], pre-diabetic [656 (463–968)] and diabetic [646 (493–817)] groups. More importantly, serum sRAGE level did not show any significant correlation with HOMA2-IR, HOMA2-%B, fasting glucose, insulin and HbA1c levels in all subjects. Serum sRAGE level also did not show any correlation with the above parameters in diabetic patients or in pre-diabetic subjects.

Conclusion: Serum sRAGE level did not show any change in pre-diabetic subjects compared with control and diabetic subjects. Moreover, sRAGE level did not show any correlation with insulin resistance or beta cell function. These data therefore suggest that sRAGE is unlikely to induce type 2 diabetes by inducing insulin resistance or beta cell dysfunction.

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PS 038 Insulin resistance and adipose tissue

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The effect of bariatric surgery on fat distribution and glucose uptake in abdominal and femoral regions in morbidly obese patients

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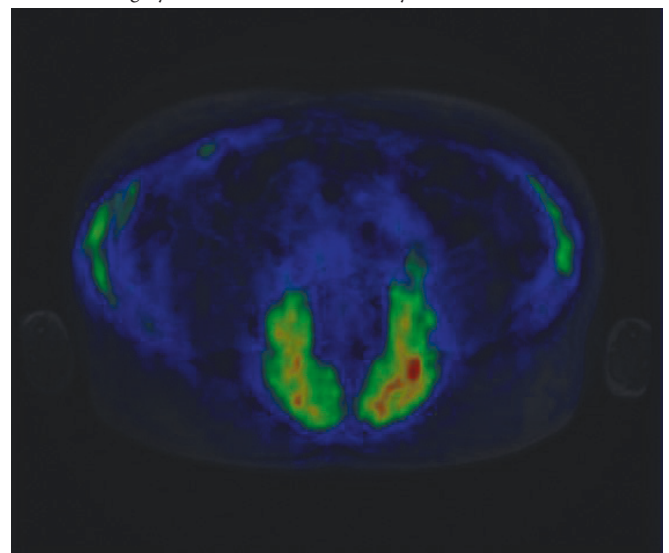
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Background and aims: Bariatric surgery reduces weight and improves insulin sensitivity. We investigated the effects of bariatric surgery on fat distribution and insulin-stimulated glucose uptake in fat tissue in abdominal and femoral regions in morbidly obese patients compared with non-obese healthy controls.

Materials and methods: Twenty five morbidly obese patients (age 46 ± 9 , body mass index 43.2 ± 3.8 kg/m^2) and 10 non-obese controls (age 47.3 ± 6.0 , BMI 23.6 ± 1.9) were studied using positron emission tomography to assess glucose uptake in fat tissue in the abdomen and in femoral area during insulin stimulation. Whole body magnetic resonance imaging was performed to measure abdominal and femoral fat content of obese and controls. Obese patients were studied before and six months after the surgery. The abdominal subcutaneous fat was divided into anterior and posterior regions. The posterior region was further divided into deep and superficial layers using the presence of fascia. The visceral fat was divided into intraperitoneal and extra-peritoneal regions using specific anatomical reference points. Subcutaneous fat mass was segmented 10 cm in the mid-section of the thigh.

Results: Total body fat (kg) in obese decreased after surgery, but remained higher compared to non-obese controls ($P < 0.001$). Compared to baseline, obese subjects lost 39% fat mass in posterior deep, 32% in posterior superficial and 40% in total anterior region ($p < 0.001$). Subcutaneous fat in the thigh decreased by 28% ($P < 0.001$). For visceral fat, intraperitoneal fat decreased by 44% while extraperitoneal fat by 29% after surgery ($P < 0.001$). In spite of the decreases in abdominal fat masses, the waist to hip ratio did not change after surgery ($P = 0.10$). Whole body insulin sensitivity increased by 94% at follow up ($P < 0.001$). Insulin-stimulated glucose uptake in abdominal adipose in deep layer increased by 19% ($P = 0.001$), 49% in superficial ($P = 0.01$) and 28% in subcutaneous fat in the thigh ($P = 0.03$) after surgery. When the various insulin-stimulated glucose uptakes were expressed per kilogram of fat, there were no changes after surgery and the results were similar to the non-obese controls.

Conclusion: Over 30% of fat mass is lost 6 months after surgery from all abdominal fat depots and adipose tissue insulin sensitivity improves in line with lipolysis. Adipose tissue metabolism in different depots remained the same after surgery and was close to the healthy controls.



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Impact of adiposity on glycaemic variability with type 2 diabetes

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Background and aims: High glycaemic variability generates oxidative stress and contributes to the development of endothelial dysfunction and cardiovascular complications in patients with type 2 diabetes. However, the factors affecting glycaemic variability have not been fully studied. In the present study, we examined the effect of body status, body composition and abdominal fat area on the parameters of glycaemic variability determined by continuous blood glucose monitoring system (CGMS) in subjects with type 2 diabetes.

Materials and methods: Among patients referred to our department between August 2012 and February 2014, 41 patients with type 2 diabetes (mean age 68.7 years; 70 % men; HbA1c 8.4 %; BMI 24.2; energy intake; 22–32 kcal/ideal body weight (60 % carbohydrate)) who underwent CGMS were selected. Their antihyperglycaemic therapy consisted of oral hypoglycaemic agents (n=31) and/or insulin (n=32) (None were diet alone). Based on CGMS data, we evaluated the following criteria for variability of glycaemic control from data of the intermediate 48 hours (midnight to midnight): average glucose levels, standard deviation (SD) values, mean amplitude of glycaemic excursions (MAGE), percentage of time spent in hyperglycaemia (> 140 mg/dL) and percentage of time spent in hypoglycaemia (< 70 mg/dL). We investigated the effect of body status (body mass index (BMI)) for these glycaemic variability indices. Furthermore, the impact of body composition (body fat mass (kg), skeletal muscle mass (kg); n=19) and of abdominal fat area (subcutaneous fat area (SFA, m²), visceral fat area (VFA, m²; n=31) on glycaemic variability is also evaluated in the subgroup analysis.

Results: Among all participants, the mean \pm SD of average glucose levels, SD of glucose variability and MAGE were 140 \pm 27 mg/dL, 32 \pm 13 mg/dL and 81 \pm 28 mg/dL, respectively. When patients were divided into two categories by median (74 mg/dL) split of MAGE, higher MAGE category had a significantly lower BMI (p=0.004) than those with lower MAGE category. In univariate analysis, BMI was significantly negatively correlated with SD (p<0.001), MAGE (p=0.015) and percentage of time spent in hypoglycaemia (p=0.005), whereas BMI was not related to average glucose levels or percentage of time spent in hyperglycaemia. In multivariate analysis relationships between BMI and MAGE or SD remained significant after adjustment of age, gender, diabetes duration and HbA1c (R²=0.29 for the model including MAGE and R²=0.41 for the model including SD), and no correlation was found with percentage of time spent in hypoglycaemia. Among body composition, body fat mass was negatively correlated to SD with a statistical significance (p=0.036), while no relationship was observed between skeletal muscle mass and SD (p=0.974). Multivariate analysis showed that body fat mass was significantly related to SD (R²=0.39) after adjustments of age and sex. Among abdominal fat area, both SFA and VFA were significantly negatively associated with MAGE (SFA; p=0.004, VFA; p=0.011) and SD (SFA; p=0.002, VFA; p=0.040). In multivariate analysis, SFA and VFA was significantly related to MAGE (SFA; R²=0.46, VFA; R²=0.50) and SD (SFA; R²=0.50, VFA; R²=0.57). **Conclusion:** In patients with type 2 diabetes, adiposity is negatively correlated with glycaemic variability independent of age, sex, diabetes duration, HbA1c or skeletal muscle mass. Our data raise the possibility that low fat mass indicates the presence of insulin secretory disorder.

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Insulin resistance predicts cardiovascular morbidity in men without diabetes mellitus and this effect is modified by level of physical activity

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Background and aims: Type 2 diabetes, as well as different measures of insulin resistance, has been shown to predict cardiovascular disease (CVD). Although type 2 diabetes is known to have a more adverse cardiovascular effect in women than in men, it is not clear whether the same is true for insulin resistance. The potentially mediating effect of physical activity on the association between insulin resistance and CVD is also yet to be fully explored. The aim of the present study was thus to assess how well insulin resistance

predicts CVD in non-diabetic men and women, and to explore the influence of physical activity.

Materials and methods: In 2002–2005, 2816 randomly selected men and women in two municipalities in Sweden, aged 30–75 years, were examined for CVD risk factors (76% participation). Anthropometric measurements were conducted, blood pressure was measured, and a standard oral glucose tolerance test was performed. Questionnaires about lifestyle and physical activity were also completed. For this prospective cohort study, 2563 subjects without diabetes and prior history of CVD were surveyed for first events of CVD through record-linkage with the National Swedish Inpatient and Mortality registers. Insulin resistance was estimated by fasting concentrations of plasma insulin and by HOMA index for insulin resistance (HOMA-IR; fasting insulin \times fasting glucose / 22.5). After controlling for proportionality, Cox proportional regression model was used to analyse the association between insulin resistance and CVD. Log transformation of HOMA-IR was used in all analyses to induce normality.

Results: After a mean follow-up of 8 years, HOMA-IR was significantly associated with increased CVD morbidity in men (50 events) and women (26 events) combined (HR 1.3, 95% CI 1.1–1.6). However, when stratified by gender, HOMA-IR was predictive for CVD solely in men (HR 1.8, CI 1.3–2.4) and not in women (HR 1.1, CI 0.1–1.5). When stratifying also for high and low physical activity the predictive value of insulin resistance became stronger in sedentary men (HR 2.3, CI 1.5–3.4), whereas it abolished in men performing moderate to vigorous physical activity (HR 1.0, CI 0.1–1.6). These results remained when also adjusting for age, APOB/APOA1, BMI, hypertension, smoking, alcohol consumption, and education, despite that almost all such risk factors showed significantly more beneficial levels in participants reporting high levels of physical activity as compared to those reporting low levels. Furthermore, a three-way interaction analysis between sex, HOMA-IR, and physical activity, was highly significant (p=0.002). The CVD risk associated with fasting plasma insulin was similar to the risk of HOMA-IR.

Conclusion: Although men may be more vulnerable to insulin resistance than women, only physically inactive men seemed to be at increased CVD risk from insulin resistance in this study. These results emphasise the importance of a high level of physical activity, as physical activity substantially increases insulin sensitivity and may also limit the negative cardiovascular effects of an increased insulin resistance in men.

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Android and gynoid fat are independently associated with hepatic and whole-body insulin resistance in non-diabetic males from the Oxford Biobank

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Background and aims: Upper- and lower-body fat depots exhibit opposing associations with insulin resistance and type 2 diabetes risk independent of overall obesity. Most associations have been detected using conventional anthropometrics, yet these measurements do not accurately quantify regional fat depots compared with techniques such as dual-energy X-ray absorptiometry (DXA). In this study we aimed to investigate whether android and gynoid DXA-quantified fat depots are independently associated with hepatic and peripheral insulin resistance in a healthy, non-diabetic, adult cohort.

Materials and methods: Subjects (406 (M) males, 387 (F) females), aged 30–50 years, were recruited from the Oxford Biobank. All subjects underwent detailed screening which included a fasting blood sample, anthropometric measurements and quantification of regional fat depots (% fat) by DXA. Plasma glucose was measured enzymatically. Plasma insulin and insulin-like growth factor binding protein 1 (IGFBP1) were measured by radioimmunoassay. Whole-body and hepatic insulin resistance were estimated using HOMA-IR and plasma IGFBP1, respectively. Spearman's Rank (r_s) and partial correlation coefficients (r) are presented.

Results: In both sexes, HOMA-IR positively correlated with android (M: 0.511, p=1.4 \times 10⁻²⁸; F: 0.501, p=4.9 \times 10⁻²⁶) and gynoid (M: 0.452, p=2.5 \times 10⁻¹⁸; F: 0.317, p=1.6 \times 10⁻¹⁰) % fat, whereas plasma IGFBP1 was inversely related to android (M: -0.612, p=3.2 \times 10⁻⁴³; F: -0.524, p=6.1 \times 10⁻²⁹) and gynoid (M: -0.443, p=4.7 \times 10⁻²¹; F: -0.248, p=7.2 \times 10⁻⁷) % fat. Partial correlation analysis identified a depot-specific relationship between % fat content and markers of insulin resistance in males. After controlling for total fat mass, android % fat remained inversely associated with IGFBP1 (-0.223, p=5.7 \times 10⁻⁶) while the association

with HOMA-IR was abolished (-0.077 , $p=0.121$). Furthermore, when HOMA-IR was corrected for, the inverse association between android % fat and IGFBP1 was retained (-0.241 , $p=9.2 \times 10^{-7}$). By contrast, gynoid % fat was inversely associated with HOMA-IR after controlling for total fat mass (-0.154 , $p=1.8 \times 10^{-3}$) and the association with IGFBP1 was abolished (0.095 , $p=0.054$). These findings could not be replicated using anthropometric measures of regional fat depots. For example, after adjusting for total fat mass, waist circumference was equally associated with HOMA-IR (0.116 , $p=1.9 \times 10^{-2}$) and IGFBP1 (-0.113 , $p=2.3 \times 10^{-2}$). In females, after controlling for total fat mass, android and gynoid % fat also displayed opposite directions of association with HOMA-IR (android: 0.124 , $p=1.4 \times 10^{-2}$; gynoid: -0.197 , $p=9.0 \times 10^{-5}$) and IGFBP1 (android: -0.145 , $p=4.2 \times 10^{-3}$; gynoid: 0.161 , $p=1.4 \times 10^{-3}$) however the strength of association was similar for both depots.

Conclusion: Our findings highlight the advantage of quantifying specific fat depots by DXA over anthropometric measures when assessing insulin resistance. In males, hepatic insulin resistance (assessed by IGFBP1) was independently associated with greater android fat content whereas whole-body insulin resistance (HOMA-IR) was associated with diminished gynoid fat content. These findings lend further support to the paradoxical relationship between diabetes risk and body fat distribution.

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A study of the mechanisms through which the biogenesis of adiponectin is regulated in white adipose tissue

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Background and aims: Adiponectin is an adipose tissue-derived hormone with anti-diabetic, anti-atherogenic and anti-inflammatory functions. Defects in adiponectin multimerization are associated with decreased adiponectin levels, obesity and insulin resistance. In this study, we investigated the enzyme systems responsible for adiponectin multimerization and production, and other factors also contributing to this process.

Materials and methods: OB mice (10- or 27-week) and age-matched C57 controls were used in this study. Fully differentiated 3T3-L1 adipocytes were also used. Quantitative real time-PCR was performed to measure the mRNA expression levels of a number of genes in white lipid tissue (WAT) from mice and 3T3-L1 adipocytes. ELISA/western blots were used to measure the expression of relative protein levels. Statistical analysis was performed by *t*-test or analysis of variance (ANOVA) with appropriate post-hoc tests.

Results: In fully-differentiated 3T3-L1 adipocytes, we observed that decreased insulin signalling caused by blocking the insulin receptor (InsR) with a blocking anti-InsR antibody, increased extracellular adiponectin levels, whereas coexisting hyperinsulinaemia counteracted this effect. Blocking the adenosine monophosphate-activated protein kinase (AMPK) pathway by using an inhibitor (compound C) significantly decreased extracellular adiponectin levels. Furthermore, we demonstrated the expression of lysyl hydroxylases (LHs), prolyl hydroxylases (PHs) and glycosyltransferase 25-domain-containing proteins 1&2 (Glt25D1&2) in the WAT of mice. Expression of LH3 was markedly increased in the WAT of OB mice compared with controls. In differentiated 3T3-L1 adipocytes, non-specific inhibition of LHs and PHs by the hydroxylase inhibitor, dipyriddy markedly suppressed adiponectin production, particularly of the higher molecular-weight isoforms, but increased the low molecular-weight isoform. Specific inhibition of prolyl-4-hydroxylase (P4H) with ethyl-3,4-dihydroxybenzoate had effects comparable to dipyriddy; however, it had different effects on the intracellular composition of adiponectin isoforms. Specific inhibition of LHs with minoxidil also suppressed adiponectin production, especially of the high molecular-weight (HWM) isoform. In addition, transient gene knock-down of LH3 (*Plod3*) suppressed adiponectin production, especially of the HMW isoform. **Conclusion:** Our results indicate that: hyperinsulinaemia and decreased insulin signalling exert countervailing effects on adiponectin production; AMPK pathway signalling also regulates adiponectin production; both PHs and LHs are required for adiponectin production; and the hydroxylation/glycosylation of lysyl residues and hydroxylation of prolyl residues are essential for formation and secretion of higher-order adiponectin isoforms.

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The plasma levels of adipokines and incretins in patients with Alzheimer's disease

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Background and aims: Alzheimer's disease (AD) is linked to type 2 diabetes (T2D). T2D is a risk factor for vascular dementia but also for a progressive neuron damage, which is probably associated with insulin resistance in central nervous system. AD is therefore sometimes called "diabetes of the brain". The aim of the study was to compare the plasma levels of adipokines, incretins and other biomarkers associated with T2D in AD patients and in non-diabetic subjects without pathological changes in the brain.

Materials and methods: The study included 38 females (19 age and BMI matched pairs AD patient and control; age 70 ± 8 years and BMI 26 ± 4 kg/m²) and 24 males (12 age and BMI matched pairs AD patient and control; age 67 ± 7 years and BMI 27 ± 3 kg/m²) who underwent neuropsychological examination and magnetic resonance imaging of the brain. The characteristics of fasting glucose and lipid metabolism and parameters of body composition including body adiposity index (BAI) were determined. Multiplex methods for evaluation of fasting plasma biomarkers were used: Bio-Plex ProHuman Diabetes 10-Plex Assay - C-peptide, ghrelin, GIP, GLP-1, glucagon, insulin, leptin, total PAI-1, resistin, visfatin and Bio-Plex ProHuman Diabetes Adiponectin and Adiponectin Assays (Bio-Rad). Statistical analyses were performed using NCSS 2004 software (ANOVA).

Results: Compared groups of AD patients and controls (men/women separately) did not differ in either age, BMI, WHR, waist and abdomen circumferences, body adiposity index (BAI) or insulin resistance (HOMAR). In women, AD patients had significantly higher levels of visfatin ($p=0.0006$), ghrelin ($p=0.03$), GLP-1 ($p=0.006$) and glucagon ($p=0.00002$) compared with control women. Similar results were found in men, they had higher levels of visfatin ($p=0.02$) and ghrelin ($p=0.02$) compared with control men, GLP-1 and glucagon did not reach statistical significance.

Conclusion: Alzheimer's disease is associated with increased levels of plasma visfatin, ghrelin, GLP-1 and glucagon. Plasma visfatin is a pro-inflammatory cytokine that increases risk of endothelial cell deterioration due to the inflammation and oxidative stress. Visfatin is a marker of ageing and age-dependent diseases. Ghrelin, GLP-1 and glucagon are considered as neuroprotective hormones. Dysregulation of these hormones in AD could be caused by impaired secretion/degradation and/or resistance of the target tissues.

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Anti-TNF therapy has a possible favorable effect on insulin sensitivity in non-diabetic, non-obese patients with inflammatory bowel disease

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Background and aims: Insulin resistance is very common in autoimmune systemic diseases and recently it was also found in children and adults with inflammatory bowel disease (IBD). Inflammation and insulin resistance are closely linked, and inflammatory cytokines such as tumor necrosis factor alpha (TNFα) may inhibit insulin signaling and promote insulin resistance. The aim of this study was to investigate the effect of anti-TNF therapy on glucose and lipid metabolism in non-diabetic, non-obese patients with IBD.

Materials and methods: We studied 41 patients with IBD (25M/16F, 36.4 ± 11 (19-64) years old, 28 with Crohn's disease and 13 with ulcerative colitis), without known history of diabetes. Eighteen patients (9M/9F, 33.6 ± 8.8 years) were on anti-TNF therapy for more than 1 year, while the other 23 patients (16M/7F, 38.7 ± 12.5 years) were treated with azathioprine and mesalazine (Aza/Mes). Nine of the patients from the second group were then treated with anti-TNF and studied again 6 months after. Fasting glucose, insulin, c-peptide, HbA1c, lipids, and CRP levels were determined and HOMA-IR index was calculated, in all patients. Statistical analysis of the data was performed using SPSS 16.00.

Results: Three of the patients were diagnosed with overt diabetes and were excluded from the analysis. Patients from the two therapy groups were

matched for age (anti-TNF: 33.6 ± 8.8 years vs Aza/Mes: 38.7 ± 12.5 years, $p > 0.05$) and BMI (anti-TNF: 23.3 ± 3.4 vs Aza/Mes: 23.1 ± 1.7 , $p > 0.05$), and were not obese. We did not find any statistical differences between the patients from the two therapy groups in the levels of fasting glucose (anti-TNF: 88 ± 10.7 vs Aza/Mes: 93.4 ± 14.9 mg/dl, $p > 0.05$), insulin (anti-TNF: 10.9 ± 7.9 vs Aza/Mes: 12.1 ± 6.6 mIU/ml, $p > 0.05$), c-peptide (anti-TNF: 1.9 ± 0.9 vs Aza/Mes: 2.2 ± 1.4 ng/ml, $p > 0.05$), HbA1c (anti-TNF: 5.2 ± 0.3 vs Aza/Mes: 5.3 ± 0.4 %, $p > 0.05$), total cholesterol (anti-TNF: 168.6 ± 32.7 vs Aza/Mes: 162.8 ± 34.3 mg/dl, $p > 0.05$), HDL (anti-TNF: 57.5 ± 15.7 vs Aza/Mes: 53.8 ± 20.3 mg/dl, $p > 0.05$), LDL (anti-TNF: 95.8 ± 28.7 vs Aza/Mes: 90.7 ± 24.4 mg/dl, $p > 0.05$), triglycerides (anti-TNF: 75.8 ± 37.6 vs Aza/Mes: 90.8 ± 61.3 mg/dl, $p > 0.05$), CRP (anti-TNF: 3 ± 5.4 vs Aza/Mes: 4.9 ± 6.1 , $p > 0.05$) and in the HOMA-IR index (anti-TNF: 2.77 ± 2 vs Aza/Mes: 3.1 ± 1.9 , $p > 0.05$). In patients who were treated for 6 months with anti-TNF, a statistically significant decrease in insulin (before: 15.4 ± 5.8 vs after: 10.2 ± 2.7 mIU/ml, $p = 0.049$) and c-peptide (before: 2.4 ± 1.2 vs after: 1.4 ± 0.4 ng/ml, $p = 0.038$) levels as well as the HOMA-IR index (before: 4.1 ± 2.1 vs after: 2.3 ± 0.7 , $p = 0.047$) was observed, without any statistically significant changes in weight, BMI, glucose, HbA1c, lipids and CRP levels (in all comparisons $p > 0.05$).

Conclusion: These preliminary data indicate that anti-TNF therapy may have a favorable effect on insulin sensitivity in non-diabetic, non-obese patients with inflammatory bowel disease.

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Dual character of osteoprotegerin as a metabolic biomarker in a healthy/prediabetic population

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Background and aims: Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily involved in bone metabolism (OPG/RANK/RANKL axis), cardiovascular (CV) system and the bone - energy homeostasis. It protects against vascular calcification in animal models while its high level has been linked to diabetic complications, CV death, inflammation and poor glycemic control in humans. However some conflicting data show that in normal glucose tolerant (NGT) subjects, these connections do not exist or show opposite results. To clarify this issue and the possible sex difference we investigated the metabolic connections of OPG in a cohort of healthy or prediabetic male and female subjects.

Materials and methods: We included 155 males and 154 females (aged 49.18 ± 9.43 vs 41.62 ± 12.95 years) in our study. They were diagnosed as NGT or glucose intolerant (GI = IGT, IFG or drug naïve 2DM) by OGTT, with no known diabetic/CV complication. IVGTT and a hyperinsulinemic euglycemic clamp were done to determine insulin secretion and sensitivity ($IS = M_m$ muscle glucose uptake). Serum glucose, insulin, free fatty acid (FFA), adipocytokines, bone markers, lipid subfractions (SF: small-dense (SD): LDL-3,4,5, large-buoyant (LB): LDL-1-2), apoproteins, anthropometric data (body fat percent, abdominal circumference (AC)) were measured. Serum OPG levels were determined by Biomedica ELISA kits.

Results: OPG levels did not differ significantly between the NGT and GI groups in either gender. In both genders it showed a significant ($p < 0.05$) positive association with TNF-alpha levels, as expected ($R = +0.178$ in males and $+0.359$ in females, both NGT and GI), which was independent of age, HbA1c and BMI. However in all males OPG has showed a significant negative correlations with BMI, FFA, triglyceride, ($R = -0.286$, -0.325 and -0.266), and positive correlation with M_m and adiponectin (AN), ($R = +0.176$ and 0.224 respectively), i.e. with improving metabolic state. The correlation with M_m ceased after correction with BMI but stayed significant with AN. In all females there was a significant ($p < 0.05$) negative correlation between OPG and FFA levels ($R = -0.359$), similar to the male group. Although the percent of LDL-3,4,5 (SD SFs) significantly increased ($R = +0.380$, 0.386 , 0.213) while that of the LDL-1 (LB-SF) decreased ($R = -0.4469$) with increasing OPG levels. These associations were not mediated by TNF-alpha because they stayed significant after data were adjusted with its levels. Feature selection analysis confirmed FFA and TNF-alpha in both genders as important determinants of OPG (mean $Z = 9.75$ and 4.12 vs 10.16 and 3.68 respectively), while SD and LB-LDL SFs were also confirmed as important attributes in females (mean $Z = 9.176$ and 2.851 , respectively).

Conclusion: Our study confirmed a gender-specific, conflicting metabolic character of OPG: it predicted improving metabolic state in both genders which was more emphasized in males. Besides, in females it predicted an

unfavourable lipid profile with increased SD and decreased LB-LDL SFs, independent of BMI or TNF-alpha levels. The nature and cause of this "dual" character of OPG has not been addressed before and needs some further prospective investigations.

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Cannabinoid receptor type 1 expression in human adipose tissue is upregulated by glucocorticoids and is associated with insulin resistance

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Background and aims: Glucocorticoids are often used for treatment of autoimmune or inflammatory conditions, including in post-transplant patients to prevent graft rejection. Elevated plasma glucocorticoid levels, as in Cushing's syndrome or during exogenous administration, are associated with a multitude of adverse effects such as obesity, dyslipidemia and insulin resistance (IR) and can eventually trigger onset of type 2 diabetes (T2D). We aimed to investigate the glucocorticoid-induced IR mechanisms in order to find unique mechanisms that could provide potential pharmacological targets or biomarkers in insulin resistant conditions.

Materials and methods: Paired subcutaneous (sc) and omental (om) adipose tissue samples were collected from non-diabetic subjects undergoing elective surgery mainly for kidney donations (M/F 10/15, 28-60 yr, BMI 20.7-30.6 kg/m²). Fasting blood samples were collected for the analysis of plasma glucose, insulin and lipid levels. In addition, anthropometric measurements were obtained. Adipose tissue was incubated for 24 h in DMEM containing 6 mM glucose and 10% fetal bovine serum in 37 °C, 5% CO₂ with or without the synthetic glucocorticoid dexamethasone (Dex) at varying concentrations (0.003-3.0 μM). Following incubation, parts of the tissue were snap-frozen for gene expression (microarray analysis, $n = 4$, with/without Dex; real time PCR, $n = 10$). Moreover, adipocytes isolated with collagenase underwent glucose uptake assays ($n = 12$) with or without insulin-stimulation (1000 μU/ml).

Results: Through affymetrix mRNA microarray analysis, it was shown that cannabinoid receptor type 1 (CNR1) is one of the genes with the highest increase in mRNA expression after incubation with a supra-physiological concentration (3 μM) of Dex in subcutaneous and omental adipose tissue (~11- and ~7-fold, respectively; $p < 0.05$). The upregulation by Dex of CNR1 gene expression was further verified by real time PCR analysis ($p < 0.05$) and shown to be dose-dependent (0.03-3 μM). Basal CNR1 gene expression in subcutaneous adipose tissue positively correlated with HOMA-IR ($p < 0.05$, $r = 0.60$), serum insulin ($r = 0.65$, $p < 0.01$) and BMI ($r = 0.56$, $p < 0.05$). In addition, CNR1 expression in subcutaneous adipose tissue treated with dexamethasone negatively correlated with basal and insulin-stimulated glucose uptake ($r = -0.74$, $p < 0.05$ and $r = -0.79$, $p < 0.01$). Furthermore, a negative correlation was seen between the Dex-induced increase in CNR1 gene expression and the basal and insulin-stimulated glucose uptake in the respective adipose tissue depots (sc basal and insulin: $r = -0.80$, $p < 0.01$; om basal: $r = -0.65$, $p < 0.05$; om insulin: $r = -0.55$, $p = 0.098$).

Conclusion: Dexamethasone promotes gene expression of CNR1 in adipose tissue, and significant associations between CNR1 expression and measures of insulin resistance were found. This study gives further support to the concept of a role of the peripheral endocannabinoid system in obesity and insulin resistance, and peripheral CNR1 may thus be an attractive target for new anti-diabetic drugs.

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PS 039 Glucagon

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Fasting hyperglucagonaemia in patients with non-alcoholic fatty liver disease with and without type 2 diabetes

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and hyperglucagonemia seems to influence glucose metabolism and both features are associated with type 2 diabetes (T2D). We investigated glucagon responses during oral and intravenous glucose administration in biopsy-verified NAFLD patients with and without T2D.

Materials and methods: We performed a 50 g-OGTT, and isoglycaemic intravenous glucose infusion (IIGI) in participants with I) normal glucose tolerance (NGT) and no NAFLD (N=10; age (median±interquartile range): 58±17 years; BMI: 29±1 kg/m²; HbA1c: 34±6 mmol/mol (5.5±0.2%)), II) NGT+NAFLD (N=9; age: 54±22 years; BMI: 28±7 kg/m²; HbA1c: 33±4 mmol/mol (5.2±0.3%)), III) T2D and no NAFLD (N=8; age: 59±18 years; BMI: 27±3 kg/m²; HbA1c: 45±9 mmol/mol (6.2±1.1%)), or IV) T2D+NAFLD (N=8; age: 65±3 years; BMI: 30±4 kg/m²; HbA1c: 53±19 mmol/mol (7.0±1.4%)).

Results: In all groups, isoglycaemia was achieved during oral and intravenous glucose administrations. Fasting plasma glucagon was elevated in participants with NAFLD and NGT (7.0±2.7 pM) and T2D (6.7±2.3 pM), compared to participants without NAFLD and NGT (2.8±4.0 pM, P<0.001) or T2D (4.5±2.9 pM, P<0.05). Increased glucagon responses (ΔAUC0-50 min) during OGTT vs. IIGI were seen in both groups with T2D (no NAFLD: 40±78 (OGTT) vs. -63±43 pM×50min (IIGI), P<0.05; NAFLD: 30±99 (OGTT) vs. -38±44 pM×50min (IIGI), P<0.05) whereas no differences in glucagon responses to OGTT and IIGI were seen in NGT subjects (no NAFLD: -20±26 (OGTT) vs. -88±60 pM×50min (IIGI), P=NS; NAFLD: -40±75 (OGTT) vs. -90±70 pM×50min (IIGI), P=NS).

Conclusion: Fasting hyperglucagonemia seems to be a pathophysiological trait of NAFLD independently of concomitant T2D. In contrast, patients with T2D have an inappropriate glucagon response to oral glucose independently of NAFLD.

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High fasting glucagon-like peptide 2 levels potentiate greater glucagon response during OGTT in patients with type 2 diabetes risk factors and newly-diagnosed type 2 diabetes

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Background and aims: The glucagonostatic action of glucagon-like peptide-1 (GLP-1) is established, whereas the influence of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-2 (GLP-2) on glucagon secretion is not fully understood. The aim of the study was to investigate the contribution of GLP-1, GIP and GLP-2 to glucagon response during the OGTT in patients with type 2 diabetes (T2D) risk factors.

Materials and methods: 127 patients with T2D risk factors (mean BMI 33.7±7.54 kg/m², mean age 57.6±12.77 years) and without prior glucose-lowering therapy underwent 75 g OGTT. Plasma glucose levels, glucagon and total GLP-1, GIP and GLP-2 levels were estimated in fasting state, at 30 and 120 minutes after glucose load and area under curve (AUC) for glucagon, GLP-1, GIP and GLP-2 response was measured.

Results: According to the OGTT results 28 subjects had normal glucose tolerance (NGT), 44 patients were either at impaired glucose tolerance (IGT) or impaired fasting glycemia (IFG) state and 55 had newly-diagnosed T2D. The stimulated glucagon secretion was suppressed in NGT group, whereas paradoxical initial increase in glucagon levels was observed in IGT+IFG and T2D patients (p<0.05). Total GLP-1 AUC was higher in NGT subjects comparing to IGT+IFG or T2D patients (p<0.01). Total GIP AUC was also higher in NGT comparing to other two groups and total GLP-2 AUC was higher in T2D group vs NGT and IGT+IFG groups, but these results were nonsignificant. When all participants were divided into tertiles by baseline total GLP-1, GIP and GLP-2 levels statistically significant difference in glucagon AUCs was reached only in patient groups divided by GLP-2 baseline lev-

els (0,008±0,064 ng/ml*min vs 0,117±0,061 ng/ml*min vs 0,638±0,065 ng/ml*min in tertiles with minimum, mean and maximum total GLP-2 fasting level, p=0,0001). Glucagon AUC was also positively related to fasting total GLP-2 level (r = 0,61, p=0,0001) in Spearman correlation analyses.

Conclusion: Positive correlation of total GLP-2 baseline levels and glucagon secretion rate suggests glucagonotropic properties for GLP-2. We conclude that glucagon stimulation by GLP-2 may play a role in the absence of glucagon suppression in T2D patients.

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The impact of autonomic neuropathy and hypoglycaemia unawareness on amino acids stimulated glucagon response to hypoglycaemia in subjects with type 1 diabetes

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Background and aims: To examine the effects of autonomic neuropathy (AN) and hypoglycemia unawareness (HU) on amino acids (AA) stimulated response of glucagon to insulin-induced hypoglycemia (H)

Materials and methods: we studied 41 subjects with type 1 diabetes (mean±SD: age 32.5±7.5 yrs; BMI 22.7±1.5 kg/m²; A1C 7.3±0.7%, diabetes duration 15.5±9.6 yrs) who were divided in three groups: no complications (ANneg, HUneg, [group A, N=15]), ANpos (group B, N=13) and HUpos (group C, N=13). Subjects were studied on two different occasions during low dose i.v. insulin (0.3 mU/kg/min) and variable glucose infusions to induce a slow hypoglycemia plateau (50 mg/dl) at 150 min maintained for 40 minutes. Then euglycemia was re-instituted and maintained for 40 minutes, until the end of study. On both occasions, at time 30 min, subjects ingested either placebo (P) or an AA mixture (42 g).

Results: In all subjects plasma insulin and glucose concentrations during the studies were not different with P and AA (p>0.1). The amount of glucose infused during the clamp was higher in the P than in the AA study (p<0.02). Plasma glucagon responses did not increase during H with P in the three groups (p=0.254). In contrast, with AA, glucagon response increased in all groups (p<0.001 vs P), less in C (p<0.05 vs A and B) although this difference disappeared after adjusting for duration (p=0.052). The linear regression analysis indicated that there was a negative correlation between diabetes duration and glucagon response to H, both with P and AA. Plasma adrenaline responses and maximal total symptom scores were not affected by AA as compared to P in all groups (p=0.621 and p=0.248, respectively).

Conclusion: AA can support responses of glucagon to hypoglycemia not only in non-complicated Type 1 diabetes, but also in conditions well known for risk of severe hypoglycemia, i.e. AN and HU. Duration of diabetes has a significant negative impact on the AA-stimulated responses of glucagon to hypoglycemia

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Glucose transporter SGLT2 inhibition triggers glucagon secretion in alpha cells

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Background and aims: Type 2 diabetes mellitus (T2D) is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, and an increase in gluconeogenesis. The gliflozins are a new class of oral antidiabetic agents acting as inhibitors of the glucose cotransporter 2 (SGLT2). By preventing renal glucose reabsorption, SGLT2 inhibition reduces the renal threshold for glucose, resulting in glycosuria. In diabetic patients, the decline in plasma glucose induced by gliflozins is, however, blunted by an increase in endogenous glucose production. Yet, the underlying mechanisms are not known. The aim of this study was to determine whether the paradoxical effect of glucagon increase in subjects with T2D might be related to a direct action on the endocrine pancreas.

Materials and methods: Human or mouse islets, alphaTC1.9 cells, human pancreatic grafts and liver from fasted mice were studied. SLC5A1/2 gene

expression was assessed by qPCR analysis and SGLT1/2 proteins by histology and ELISA. RNA interference or SGLT inhibitors such as phlorizin or dapagliflozin was used to inhibit SGLT2 in alpha cells, human islets or healthy mice.

Results: Here, we demonstrate that SGLT1/2 proteins (encoded by the SLC5A1/2 genes) are specifically expressed in human pancreatic alpha cells. SLC5A2 gene expression is lower in islets isolated from T2D patients ($p<0.05$) and decreases in normal human islets after transplantation in insulin resistant immuno-deficient mice ($p<0.05$). Lower SLC5A2 gene expression is accompanied by an increase of SLC5A1 ($p<0.01$) and glucagon (GCG) gene expression ($p<0.001$). siRNA knockdown of SGLT2 in alpha cells, or its inhibition with phlorizin or dapagliflozin stimulated GCG gene expression ($p<0.001$) and glucagon secretion ($p<0.05$). In healthy mice, dapagliflozin treatment elevated fasting plasma glucose levels ($p<0.001$) and hepatic gluconeogenic gene expression (G6pase and Pepck) ($p<0.05$).

Conclusion: Collectively, these results reveal that inhibition of SGLT2 activity induces glucagon secretion, identifying a hereto unknown role of glioflozins as alpha cell secretagogues.

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Paradoxical synchronisation of Ca^{2+} oscillations between alpha and beta cells within intact pancreatic islets

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Background and aims: Insulin and glucagon are secreted in anti-synchronous pulses causing pronounced variations in the insulin/glucagon ratio that determines hepatic glucose production. The insulin pulses are generated by oscillations of the cytoplasmic Ca^{2+} concentration, which is regarded the main trigger of both insulin and glucagon release. To clarify how the glucagon release pattern is generated it is important to understand the relationship between the cytoplasmic Ca^{2+} dynamics in α - and β -cells.

Materials and methods: Pancreatic islets were isolated from transgenic GLU-RFP mice expressing red fluorescent protein (RFP) in the α -cells. The Ca^{2+} concentration in the sub-plasma membrane space ($[\text{Ca}^{2+}]_{\text{pm}}$) of superficial islet cells was recorded with total internal reflection microscopy and the fluorescent indicator Fluo-4.

Results: Introduction of 1 mM glutamate in the presence of the hyperpolarizing agent diazoxide triggered a pronounced elevation of $[\text{Ca}^{2+}]_{\text{pm}}$ in 84% of the RFP-positive cells but had no effect in the RFP-negative ones, suggesting that glutamate responses can be used as a complementary tool to distinguish α - from β -cells. At 3 mM glucose, α -cells showed pronounced, unsynchronized $[\text{Ca}^{2+}]_{\text{pm}}$ oscillations, whereas $[\text{Ca}^{2+}]_{\text{pm}}$ was low and stable in the β -cells. When the glucose concentration was increased to 20 mmol/l, β -cells showed an initial decrease of $[\text{Ca}^{2+}]_{\text{pm}}$ followed by a protracted increase, succeeded by oscillations that were almost perfectly synchronized among neighbouring β -cells. The α -cell oscillations were often temporarily suppressed in parallel with the initial $[\text{Ca}^{2+}]_{\text{pm}}$ increase in the β -cells. When subsequently recurring, the α -cell oscillations tended to synchronize with those in the β -cells, a phenomenon consistently evident when averaging Fluo-4 fluorescence from several α -cells within an islet.

Conclusion: Pancreatic α - and β -cells show distinguishing $[\text{Ca}^{2+}]_{\text{pm}}$ signaling patterns in low and high glucose and in response to glutamate. Synchronization of $[\text{Ca}^{2+}]_{\text{pm}}$ oscillations between α -cells and β -cells at 20 mM glucose surprisingly indicates that pulsatile glucagon release is not generated by α -cell oscillations of $[\text{Ca}^{2+}]_{\text{pm}}$ unless Ca^{2+} becomes an inhibitory messenger under these conditions.

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Possible contribution of taurine to distorted glucagon secretion in insulin deficiency: a metabolome analysis using a novel alpha cell model of type 1 diabetes

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Background and aims: Glycemic instability is a serious problem in patients with type 1 diabetes (T1D). We recently reported that glucagon secretory capacity is positively associated with the degree of glucose fluctuation in T1D patients whose endogenous insulin was completely depleted, suggesting that glycemic instability may be due in part to paradoxically increased endogenous glucagon secretion. However, the intracellular metabolic mechanism(s) involved in the aberrant glucagon response under the condition of insulin deficiency has not yet been elucidated.

Materials and methods: We generated an α TC6 cell line with lentiviral shRNA-mediated stable knockdown of the insulin receptor (IRKD), i.e., an *in vitro* α -cell model for T1D. The glucagon secretion in response to varying glucose concentrations (1.5, 5.6 and 30 mM) was studied in both control and IRKD cells. To investigate the cellular metabolic changes in α -cells under pathophysiological conditions of T1D, a comprehensive intracellular metabolomic analysis of the IRKD α TC6 cells (IRKD cells) was performed using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). The various metabolites, which were identified as being significantly altered in the IRKD cells and could potentially affect the glucagon secretion, were chosen for further uptake and biosynthetic analyses.

Results: The IRKD cells tended to secrete less glucagon compared to the controls at the 1.5 mM glucose concentration ($p=0.13$). On the other hand, the IRKD cells exhibited significantly higher glucagon secretion in response to the 30 mM glucose concentrations than controls (60.1 ± 4.2 vs. 18.0 ± 1.1 pg/ μ g protein, $p<0.01$). By metabolome analysis, the cellular contents of 33 metabolites in the control and IRKD cells were significantly different, with $p<0.05$ for at least two points at each concentration of glucose. Of these metabolites, taurine was remarkably increased in the IRKD cells and was one of the most likely candidate metabolite stimulating glucagon secretion. The cellular uptake of [³H] taurine was increased time-dependently in IRKD cells compared to control cells. The expression of cysteine sulfinic acid decarboxylase (*casd*), the rate-limiting enzyme required for taurine synthesis, was increased in IRKD cells compared to control cells ($p<0.05$). Supplementation of taurine (100 mM) to the culture medium enhanced the glucagon secretion in a concentration-dependent manner in the α TC6 cells. When the IRKD cells were treated with 30 mM glucose plus 10 mM taurine, the glucagon secretion was significantly augmented compared with that in vehicle-treated cells (63.3 ± 2.5 vs. 49.1 ± 0.9 pg/ μ g protein, $p<0.01$).

Conclusion: Our study indicates that the metabolic alterations induced by IRKD in α -cells, especially the increase of taurine, may lead to a distorted glucagon response in IRKD cells, suggesting the importance of taurine in the paradoxical glucagon response and the resultant glucose instability in T1D.

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Expression and function of the atypical purinergic receptor GPR17 in endocrine pancreas

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Background and aims: Islets of Langerhans control glucose homeostasis and metabolism by sensing blood glucose and nutrients. Factors secreted within the islet create a parallel tuning, defined autocrine-paracrine signaling, that modulates hormone release and cell viability. Studying the molecular actors of this signaling and their physiological significance may help in understanding diabetes pathogenesis thus providing novel therapeutic targets. G-protein coupled receptors (GPCRs) are expressed in islets of Langerhans, regulate hormone secretion and cell survival, and are emerging as new targets for type-2 diabetes therapies. In this work, we focused on the expression and

role of the atypical orphan receptor GPR17, which is activated by uracil nucleotides and can be partially antagonized by ATP, a well known autocrine/paracrine signal in islets. We first verified the GPR17 expression in endocrine cell lines and in human islets, and then we investigated its possible role in islet physiology

Materials and methods: The expression of GPR17 was analysed by RT-PCR analysis, western blot and immunofluorescence techniques in cell lines (α TC3, β TC3, the δ cell line RIN14B), in human isolated islets of Langerhans, and human pancreatic sections. The possible involvement of GPR17 in the control of hormone release and cell proliferation/viability was studied in cell lines and in human isolated by means of ELISA assays.

Results: By means of RT-PCR, we detected GPR17 mRNA in human islets, in α and δ cell lines, but not in β cell lines ($n=5$). By means of western blot experiments, we confirmed GPR17 protein expression in α and δ cell lines and in human islets, where its abundance was upregulated by hyperglycemia (2.13±0.15 fold increase over normoglycemic conditions; $p<0.05$, $n=4$). To investigate the specific endocrine cells where GPR17 was expressed *in vivo*, we carried out double immunofluorescence and confocal analysis on human pancreas sections, using hormones as markers of the different cell populations. We confirmed the expression of GPR17 in a sub-population of δ cells but no expression was detected in β -cells, in physiological conditions. To understand the possible role of GPR17 in islet physiology we activated GPR17 with its agonist UDP-glucose, and we measured its effect on cell viability and hormone release in cell lines and human isolated islets. 3-day incubation of α TC3 with 100 μ M UDP-G caused a significant increase in cell viability, measured by MTT test (108±2.56% UDP-G vs ctr, $p<0.005$ T-Test; $n=10$). A similar behavior was observed in human islets, where we found a reduction of apoptosis in presence of 100 μ M UDP-G (38±7% UDP-G vs ctr). Preliminary data on hormone release in static conditions indicated that incubation of human islets with 100 μ M UDP-G, caused a decrease in the glucagon and somatostatin release in normoglycemia (37±10% and 25±5%, respectively) (Elisa assay, 2 islets preparations, duplicate)

Conclusion: We demonstrated for the first time that GPR17 is expressed and functional in human islets of Langerhans. Studies are in progress to better define its role in islet cell physiology and pathology.

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Elevation of cAMP promotes an Epac2 dependent, L-type Ca^{2+} -channel coupled, Ca^{2+} -induced Ca^{2+} release in mouse alpha cells

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Background and aims: Glucagon is a potent glucose elevating hormone that is secreted from pancreatic α -cells in response to hypoglycaemia. Glucagon secretion in mouse islets is inhibited by elevation of glucose and strongly stimulated by adrenaline. Although glucose and adrenaline have differential effects on glucagon secretion, these two different factors exert similar effects on α -cell electrical activity. This indicates that the stimulatory effect of adrenaline is exerted distal to the firing of action potentials, i.e. cell exocytosis. Cell exocytosis is Ca^{2+} -dependent and relies on Ca^{2+} from the extracellular environment or released from intracellular Ca^{2+} stores (by a process namely Ca^{2+} -induced Ca^{2+} release). cAMP is known to regulate several pathways in Ca^{2+} signaling via phosphorylation by protein kinase A (PKA) and/or Epac (exchange protein activated by cAMP). This study aims to address whether this amplifying pathway is governed by cAMP in pancreatic α -cells.

Materials and methods: Mouse pancreatic islets were acutely isolated by enzyme digestion. Hormone secretion was measured by batch incubation experiments. Ca^{2+} signal was ratiometrically monitored using indo-1. Cell exocytosis was measured using capacitance measurements. Membrane potential was measured using intact mouse islets with perforated patch clamp technique under current clamp mode.

Results: Adrenaline (5 μ M) stimulated glucagon secretion 2 fold, an effect that was also produced by 10 μ M of the adenylyl cyclase activator forskolin ($n=8$). Adrenaline-stimulated glucagon secretion can be reversed following application of the L-type Ca^{2+} channel blocker isradipine (2.5 μ M) or 100 μ M ryanodine (an intracellular Ca^{2+} releasing channel blocker). α -cell exocytosis evoked by 300ms depolarization (from -70mV to 0mV) was strongly stimulated when 0.1mM cAMP was applied intracellularly; from 37±9fF ($n=16$) to 233±32fF ($n=17$). This was accompanied by a marked increase in Ca^{2+} transients (AUC from 1.4±0.3 to 2.5±0.8, $p<0.05$). cAMP delayed the decay of depolarization evoked Ca^{2+} transients (time constant τ , from 0.6±0.1s to 1.7±0.5s, $p<0.05$). Neither the amplitude, nor the decay of Ca^{2+} transient were

reduced when the PKA inhibitor Rp-CAMPS or PKI were applied in the presence of cAMP. In α -cells from mice lacking Epac2 (Epac2^{-/-} mice), the cAMP amplification of cell exocytosis was markedly reduced to an insignificant level (from 32±7fF to 53±12fF; $n=16$ for control and $n=8$ for cAMP, $p=0.1$). Furthermore, cAMP did not produce a significant change in the kinetics of depolarization triggered Ca^{2+} transients in epac2^{-/-} α -cells. Adrenaline exerted a weaker stimulatory effect on glucagon secretion in epac2^{-/-} mouse islets (2 fold compared to 5 fold in wildtype, $n=8$). The PKA inhibitor Rp-cAMPS partially reduced adrenaline stimulated glucagon secretion in wildtype islets, but completely abolished that in epac2^{-/-} islets.

Conclusion: Adrenaline stimulates glucagon secretion in mouse islet α -cells via a cAMP /Epac2 dependent pathway. This effect is likely to be exerted distally to electrical activity by promoting L-type Ca^{2+} channel coupled Ca^{2+} -induced Ca^{2+} release from the endoplasmic reticulum via ryanodine receptor. This study provides a novel insight into the regulation of glucagon secretion by adrenaline and highlights the importance of appropriate neuronal control in preventing life-threatening episodes of hypoglycaemia in diabetes.

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Alpha cell derived glucagon-related peptides are required for normal beta cell function and glucose homeostasis

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Background and aims: Recent data indicate that pancreatic alpha cell derived glucagon-like peptide-1 (GLP-1) and glucagon contribute to beta cell function. We studied the importance of these locally acting hormones versus systemically derived insulin secretagogues on beta cell function and glucose homeostasis.

Materials and methods: Selective and massive alpha cell ablation was achieved by diphtheria toxin injection into mice with alpha cell specific expression of human diphtheria toxin receptor at the age of 5 weeks. *In vivo* intraperitoneal glucose tolerance tests, and *in vitro* glucose-stimulated insulin secretion assay on isolated islet were performed.

Results: In agreement with previously published data, we did not observe any difference in intraperitoneal glucose tolerance between alpha cell ablated mice and their littermate controls up to the age of 12 weeks. At the age of 28 weeks, alpha cell ablated mice had impaired intraperitoneal glucose tolerance and lower insulin secretion compared to littermate controls despite similar baseline systemic active GLP-1 levels (3.6 ± 0.9 pg/ml in alpha cell ablated mice versus 3.0 ± 0.8 pg/ml in littermate controls). The ileum content of active GLP-1 was compensatory increased by 32% ($p = 0.06$) in alpha cell ablated mice. Body weight was comparable in both groups. Both sitagliptin injection to stabilize systemic GLP-1 or glucagon injection prior to intraperitoneal glucose load restored glucose tolerance in alpha cell ablated mice. In isolated islets from alpha cell ablated mice, glucose-stimulated insulin secretion was reduced by 69% ($p < 0.0001$) compared to wildtype islets. Administration of exogenous GLP-1 or glucagon as well as the adenylyl cyclase activator forskolin during high glucose stimulation fully restored insulin secretion *in vitro*.

Conclusion: Alpha cell derived GLP-1 and glucagon are required for normal glucose homeostasis and beta cell function *in vivo*. Further, these findings indicate that the presence of cyclic AMP mediated beta cell priming by local glucagon-related peptides is crucial for full glucose-stimulated insulin secretion.

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PS 040 Exercise physiology I

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Improvement in insulin sensitivity following exercise training is independent of mitochondrial changes in older adults

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Background and aims: Previous studies reported lower skeletal muscle mitochondrial oxidative capacity as one of the links between type 2 diabetes and aging. This gave rise to the interesting indication that mitochondrial deficiency may be an important factor in the pathogenesis of insulin resistance. We have recently shown that exercise training improves mitochondrial content and function in older sedentary adults. The purpose of this study was to link these improvements with improvements in insulin sensitivity.

Materials and methods: Twenty sedentary older (age range 61 to 72) men and women (11M, 9F), with average BMI $28 \pm 4 \text{ kg/m}^2$ and either impaired or normal glucose tolerance (11 IGT, 8 NGT), participated in a four months supervised endurance exercise intervention with a pre/post-intervention design. Investigations included glucose clamps to measure insulin sensitivity, dual X-ray absorptiometry for overall body composition, magnetic resonance imaging for visceral adipose tissue (VAT) and graded exercise testing for maximal oxygen consumption ($\text{VO}_{2\text{max}}$). Maximal ATP production capacity (ATPmax) was used as marker for mitochondrial function and was estimated by phosphocreatine recovery following exercise using ³¹P magnetic resonance spectroscopy. *Vastus lateralis* muscle biopsies specimen were used to measure mitochondrial content by electron microscopy and western blotting. Transcription factors involved in mitochondrial biogenesis were measured in a subset of volunteers by quantitative RT-PCR.

Results: The following changes were observed with intervention (data are mean change \pm SEM): insulin sensitivity (glucose infusion rate, GIR) $+41 \pm 6\%$ ($P < 0.001$), BMI $-1.6 \pm 0.7\%$ ($P = 0.02$), body fatness $-7.3 \pm 1.6\%$ ($P < 0.001$), VAT $-6.3 \pm 2.8\%$ ($P = 0.04$), $\text{VO}_{2\text{max}}$ $+11.7 \pm 3.5\%$ ($P < 0.001$), ATPmax $+18.7 \pm 6.3\%$ ($P = 0.02$), mitochondrial volume density (MitoVd) $+54.9 \pm 11.8\%$ ($P < 0.001$), PGC1 α mRNA $+84.5 \pm 36.3\%$ ($N = 12$, $P < 0.001$) and TFAM mRNA $+60.6 \pm 30.6\%$ ($N = 6$, $P = 0.04$). Significant differences in these responses were not observed between IGT vs. NGT, neither between obese vs. lean volunteers. Although GIR correlated with ATPmax and MitoVd at baseline ($P < 0.05$), changes in insulin sensitivity were not associated to changes in ATPmax nor MitoVd.

Conclusion: In this cohort of older subjects, four months of exercise intervention significantly enhanced skeletal muscle mitochondrial content and function. Insulin sensitivity was markedly improved in the whole cohort, as well as in both IGT and NGT volunteers. Interestingly, the improvements in insulin sensitivity were not related to the increases of mitochondrial content or function. Although the existence of mitochondria dysfunction may worsen insulin resistance, the lack of relationship between improvements in both mitochondria and insulin sensitivity questions the function of mitochondria on insulin resistance. Further studies are needed to elucidate the debated pathogenic role of mitochondria in insulin resistance of aging. In other terms is mitochondrial dysfunction a cause or a consequence of insulin resistance?

Clinical Trial Registration Number: NCT01224886

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Endurance exercise training reduces inflammation and apoptosis of the hypothalamic neurons in obese mice

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Background and aims: Consumption of fat-rich diets blunts leptin and insulin anorexigenic signaling in the hypothalamus by a mechanism dependent on the in situ activation of inflammation. Since inflammatory signal transduction can lead to the activation of apoptotic signaling pathways, we evaluated the effect of endurance exercise training on the reduction of apoptosis of hypothalamic cells.

Materials and methods: Swiss mice was divided into 6 groups (with 5-8 each): Control Group 1 and 2 (C1 and C2): sedentary animals fed with balanced diet for 8 or 16 weeks, respectively; Obese 1 and 2 (OB1 and OB2): sedentary animals fed with high-fat diet (HFD) for 8 or 16 weeks, respectively; Trained obese 1 (OBT1): animals fed HFD for 8 weeks and submitted to physical training protocol (PTP) during 8 weeks; Trained obese 2 (OBT2): animals fed HFD for 16 weeks and submitted to PTP during the second half of the experiment (8 weeks). The exercise program consisted of treadmill running 1h, 5 days/week during 8 weeks at a speed equivalent to 60% of maximum potency determined at the beginning of training period. For determination of food intake, mice were intracerebroventricular treated with insulin or leptin. Twenty-four hours after the last bout exercise, the mice were submitted to an insulin tolerance test. Next, the hypothalamus was removed and Western blotting and immunoprecipitation were performed to analyze the expression and phosphorylation of IR, IRS-2, Akt, JAK, STAT3, Foxo1, JNK, IKK, Bax, Casp9, Casp8, FADD, APAF, PERK, eIF2 α .

Results: PTP was effective in improving the IR/IRS-2/Akt and JAK2/STAT3 signaling pathway in the hypothalamus and reduced food intake of obese animals. The HFD led to an increased hypothalamic expression of proteins involved in inflammatory signal transduction (pJNK and pIKK). In addition, proteins from apoptotic pathways were affected by HFD. Expression of Bax and the association of APAF1 with caspase-9, and the association of FADD with caspase-8 were increased in the hypothalamus of obese mice. Further evidence for apoptotic or harmful activity in the hypothalamus was shown by the increased expression of PARP1, and by the phosphorylation of proteins involved in endoplasmic reticulum stress, eIF2 α and PERK. Oppositely, PTP was able to reduce inflammatory and apoptotic proteins in the hypothalamus of these animals. Interestingly, the results obtained were more pronounced in mice of OBT1 than OBT2. This shows that the effects of exercise were more effective when the inflammatory and apoptotic process were absent.

Conclusion: Our study provides substantial evidence that physical activity could help to reorganize the set point of nutritional balance and therefore aid in counteracting the energy imbalance induced by overnutrition through the anti-inflammatory and anti-apoptotic response in hypothalamic neurons.

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The effect of chronic exercise training on exercise efficiency and fat oxidation in older previously sedentary adults

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Background and aims: Chronic aerobic exercise has been shown to increase exercise efficiency, which allows us to spend less energy for a similar amount of work. Exercise efficiency can be expressed as gross efficiency, computed as mechanical power (wattage) over the metabolic rate (calories expended); thus, representing whole-body efficiency. Other efficiencies are also used such as delta efficiency, which is the change in power over the change in metabolic rate between different intensities of exercise. Furthermore, it is known that substrate usage during exercise also changes with chronic training to rely more on fat than carbohydrates for a given submaximal exercise intensity. All

of these training adaptations have not yet been explored in older subjects at risk for type 2 diabetes. The purpose of this study was to examine the changes in exercise efficiencies and exercise fat oxidation in older sedentary adults undergoing an exercise intervention and compare these values to athletes of the same age.

Materials and methods: In this pre/post intervention design, 11 sedentary obese (age 67.0 ± 1.1) and 12 sedentary lean (age 64.6 ± 1.0) men and women underwent four months of endurance exercise intervention. Volunteers trained 3 supervised sessions per week at 60–75% of their maximal heart rate. Fifteen athletes matched by age (age 68.5 ± 1.5) were also used for comparison purposes. Peak oxygen consumption ($\text{VO}_{2\text{peak}}$) was determined by a graded exercise testing (GXT). Gross efficiency (GE, %) and exercise fat oxidation (EFO, $\mu\text{mol}/\text{min}$) were determined during one-hour of submaximal (55% of $\text{VO}_{2\text{peak}}$) cycle ergometry exercise in tightly controlled conditions (fasting and 72 hours from last exercise bout). Delta efficiency (DE, %) was calculated from the GXT. Leg mass, used to normalize efficiency data, was measured by dual X-ray absorptiometry. Statistical comparisons between groups at baseline were done with ANOVA. Intervention effects were measured by Paired T Test. Results are presented in mean \pm SEM.

Results: At baseline, athletes had a higher GE than lean sedentary, which had a higher GE than the obese sedentary ($p < 0.001$). Both the obese and lean increased their GE after four months of exercise training (respectively $p = 0.0012$ and $p = 0.001$). DE followed the same pattern as GE at baseline ($p < 0.001$). Also similar to GE, both obese and lean increased their DE following exercise intervention (respectively $p < 0.001$ and $p = 0.001$). EFO was the same between all three groups at baseline ($p = 0.35$). EFO did not change in the lean sedentary group ($p = 0.47$) after the intervention but did increase in the obese ($p = 0.01$).

Conclusion: This data shows that older adults are able to respond to a moderate intensity exercise training protocol in terms of improvements in GE and DE, as shown in younger and performance-oriented studies. Thus, they expend fewer calories for the same power output. These observations point to the fact that exercise routines need to be modified in order to elicit metabolic adaptations and keep optimal caloric expenditure also at an older age. Former studies show that training improves the reliance on fat. Surprisingly, in our cohort fat oxidation was not different between the three groups at baseline. The obese group was the only one to improve fat oxidation after four months of exercise training, which can be explained by changes in body composition. *Clinical Trial Registration Number: NCT01224886*
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Changes in muscle lipid stores after exercise training in subjects with prediabetes and healthy controls

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Background and aims: There is an association between muscle lipid stores and insulin sensitivity, but the mechanism is unclear. We examined how exercise intervention influenced muscle lipid stores in sedentary normal-weight control subjects and overweight prediabetic subjects impaired fasting glucose or impaired Glucose tolerance.

Materials and methods: Ten sedentary male control subjects and eight sedentary male prediabetic subjects participated in 12 weeks supervised training. Insulin sensitivity (euglycaemic clamp) and $\text{VO}_{2\text{max}}$ were measured. Muscle lipid stores were quantified by magnetic resonance spectroscopy (MRS), electron microscopy (EM) point counting, and direct EM lipid droplet measurements of subsarcolemmal (SS) and intermyofibrillar (IMF) regions, and indirectly, by deep sequencing and real-time PCR of mRNA of lipid droplet-associated proteins.

Results: Insulin sensitivity (+28% and +36%) and $\text{VO}_{2\text{max}}$ (+14% and +15%) increased significantly in both the prediabetic and the control groups after 12 weeks of training. Muscle lipid stores were reduced, as measured by MRS at baseline before and after the intervention, whereas EM point counting showed no change in LD stores post-exercise, indicating a reduction in LD store utilization with lowered LD levels. Large scale EM quantification of the sub-sarcolemmal LD population demonstrated reductions in LD density and LD diameters. The SS LD diameters were reduced by 28% and 26%, and SS LD numbers were reduced by 37% and 43%, for the prediabetic and the control groups, respectively. Thus, lipid droplet volume in the SS LD popu-

lation was reduced by ~80%, in both prediabetics and normal weight controls. Interestingly, the lipid droplet diameters ($n = 10\,958$) distribution was skewed, with a lack of small diameter lipid droplets (smaller than ~200 nm), both in the SS and IMF regions.

Conclusion: We demonstrate reduced muscle lipid stores in the control and prediabetic groups, using MRS, but no corresponding differences post-exercise, using EM point counting, suggesting reduced LD utilization with lower LD stores. A large-scale EM quantification of LD numbers and diameters showed a differential behavior of two populations of LD. The SS LD were reduced by ~80% in both groups, whereas the intramyofibrillar population of LD did not show any consistent changes. The EM quantification provided a skewed distribution of lipid droplet size, with a lack of small (< 200 nm) diameter LDs both in the SS and IMF regions, before as well as after the intervention.

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The improvement in beta cell function after intense aerobic exercise is directly related to the increase in vitamin D levels

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Background and aims: Low concentrations of 25-hydroxy-vitamin D [25(OH)D], the best indicator of vitamin D status, have been associated with alterations in glucose tolerance, insulin sensitivity and β cell function. Few data are available on the effects of a single session of aerobic exercise on 25(OH)D levels and the possible correlation with insulin secretion and sensitivity.

Materials and methods: We therefore recruited 20 young (25–31 years-old), healthy, normal weight volunteers, with a sedentary/moderate active lifestyle; OGTT was performed before and on the day-after a bout of aerobic exercise (a single workout of jogging or running for 30–40 minutes, or until exhaustion). Insulin secretion and sensitivity were estimated using OGTT-derived indices. During exercise, all subjects wore a metabolic holter to assess energy expenditure.

Results: Based on the energy expenditure during work-out, subjects were divided (the median in the cohort [7 METs] was used as cut-off value) into: non-intense (<7 METs) or intense group (>7 METs). In both groups, a significant improvement in insulin sensitivity ($p < 0.04$ Quicki) was observed. After exercise, 25(OH)D levels increased in the intense group and decreased in the non-intense group ($p = \text{NS}$ for both). Furthermore, 25(OH)D levels, as well as the disposition index, were significantly greater in the intense group after physical activity ($p < 0.02$ and $p < 0.03$, respectively), as compared with the non-intense group. The improvement in the disposition index was correlated with the change from baseline in 25(OH)D in the intense group ($r = 0.4$, $p < 0.05$).

Conclusion: These data indicate that a single session of aerobic exercise might improve beta cell secretory performance and this improvement is closely related with the increase in the levels of 25(OH)D after strenuous physical activity.

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Metabolic effects of interval training exercise in patients with type 2 diabetes

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Background and aims: Major guidelines on the management of type 2 diabetes mellitus (T2DM) recommend performing at least 150 min/week of moderate-intensity aerobic physical activity. Nevertheless, the most effective type of exercise for glycaemic control has not been established. The aim of the present study was to compare the effects of interval training exercise, which pairs high-intensity exercise phases with low-intensity exercise recovery phases, with those of a conventional, constant-load aerobic exercise on glycaemic control (HbA1c), lipid profile and body composition in patients with T2DM.

Materials and methods: Thirty sedentary patients (17/13 M/F; age [mean \pm SD] 63.0 ± 8.0 yrs; BMI 29.0 ± 3.9 kg/m²; HbA1c% [median (1st–3rd quartile)] 6.6 (6.5–7.1) with a diagnosis of T2DM ≥ 5 years and treated with oral hypoglycaemic agents and/or basal insulin were randomized into 3 groups: constant-load aerobic exercise (CL), interval training aerobic exer-

cise (IT) for 10 weeks (a 2-week conditioning phase + 8-week exercise programme, 3 times/week) or no activity (SED) for the same time period. Fasting plasma glucose, insulin, HbA1c, triglycerides (TG), total cholesterol, HDL and LDL cholesterol and body composition (DXA) were measured at baseline and at study end.

Results: Baseline characteristics of the 3 groups were comparable. After 10 weeks, there were no significant differences from baseline in HbA1c% [pre vs. post: 6.7 (6.5–7.1) vs. 6.6 (6.5–6.9); 6.6 (6.5–7.0) vs. 6.5 (6.5–6.6); 6.7 (6.1–7.5) vs. 6.6 (6.4–7.2) in the CL, IT and SED groups, respectively; p for time and interaction=ns] or cholesterol levels. Significant improvements in fasting plasma glucose ($p=0.03$), TG ($p=0.02$) and insulin resistance estimated with the HOMA-IR index ($p=0.04$) were observed in the IT group only, despite similar and significant changes in total body composition in both exercise groups (fat mass: -4.3% and -4.9%, fat free mass +1.5% and +1.7% in the CL and IT groups, respectively; both $p<0.05$ vs. baseline; CL vs. IT: $p=ns$).

Conclusion: Interval training exercise might have specific effects on segmental body composition and/or lead to different adaptive changes in skeletal muscle as compared with constant load aerobic exercise. Such changes might be responsible for the observed benefits on fasting plasma glucose, TG and HOMA-IR. The lack of significant effects on HbA1c might be due to the good glycaemic control at baseline.

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Investigation of glycaemic control in elite and recreationally-active individuals with and without type 1 diabetes mellitus during multi-day ultra-distance exercise: 2014 mHealth grand tour

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Background and aims: Despite improvements in cardiovascular health and wellbeing, fear of exercise-induced hypoglycaemia and a paucity of evidence-based advice on how to manage diabetes during / after exercise remain significant barriers to exercise participation to people with Type 1 diabetes (T1DM). How professional athletes with T1DM manage their diabetes may provide an insight into optimising diabetes control around exercise. The present study examined glucose control in elite and recreational cyclists with and without T1DM during participation in the 2014 mHealth grand tour cycle ride from Brussels to Barcelona (EASD).

Materials and methods: Interstitial glucose (IG) concentrations were captured using continuous glucose monitoring (Dexcom G4[®], INC, CA, USA) over a period of six consecutive days during a long-distance, multi-stage endurance cycling event in three groups of individuals: 10 recreationally-active type 1 diabetes individuals (T1rec), 8 elite cyclists with T1DM (T1pro), and 10 recreationally-active individuals without diabetes (CON). Each individual completed all 6 days of road cycling in full, covering a total distance of 1044km (174km av/day). Data (mean±SD) were analysed using a one-way ANOVA and independent student t tests.

Results: There was no change in glycaemic control over the course of the six days in any group, with similar mean, mean peak and mean nadir IG during ride time, daytime and night time periods ($p>0.05$). During the ride, mean IG was significantly greater in T1rec and significantly lower in T1pro compared to CON (7.6±2.8 vs. 5.2±2.7 vs. 6.3±0.1 mmol.l⁻¹; $p<0.05$). During the ride, time spent in hypo- and hyperglycaemia were similar between T1rec and T1pro (hypo: 209±49 vs. 207±57min; $p=0.332$; hyper 240±5 vs. 244±10min; $p>0.05$). During recovery time before sleep T1pro displayed similar mean IG to CON (6.4±2.8 vs. 6.6±0.9 vs. mmol.l⁻¹; $p=0.553$), which was significantly less than T1rec (9.3±1.7 mmol.l⁻¹; $p=0.013$). Similar patterns were observed during sleep, whereby T1pro displayed similar mean IG to CON (T1pro 6.7±3.4, CON 6.1±0.9 mmol.l⁻¹; $p=0.412$), which were both significantly less than T1rec (8.7±2.6 mmol.l⁻¹; $p<0.05$). However, more T1rec individuals experienced nocturnal hypoglycaemia compared to T1pro (T1rec 66% vs. T1pro 13% patients) with more time spent hypoglycaemic (T1rec 402±62, T1pro 223±51 minutes; $p=0.018$) and lower mean IG nadir under T1rec (T1rec 2.6±1.3, T1pro 4.3±2.5, CON 4.4±0.7 mmol.l⁻¹; $p<0.05$) during this time.

Conclusion: Recreational cyclists with T1DM had higher IG levels during exercise, in recovery and during sleep, and experienced more hypoglycaemic events than elite athletes with T1DM. Elite athletes with T1DM displayed IG comparable to people without diabetes during exercise, in recovery and during sleep. Glycaemic stability in elite athletes with T1DM provides a valuable insight to strategies that can be used to optimise glycaemic control during exercise in the wider T1DM patient population.

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The effect of basal insulin dose reduction during exercise in adolescents with type 1 diabetes mellitus

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Background and aims: Physical activity helps improve metabolic control and reduces insulin requirement in type 1 diabetic children (T1DM). There is no consensus regarding the guidelines for administration of insulin in patients treated with continuous subcutaneous insulin infusion during physical activity. The aim of the study was to assess the impact of basal insulin reduction on the risk of hypoglycemia in T1DM children treated with insulin pump.

Materials and methods: We performed a non-randomized, open-labeled, two-way crossover study. There were 50 patients included, 28 (58%) boys and 22 (42%) girls, aged 14.5 ± 2.3 years (range 10–18) with mean diabetes duration 7.1 ± 3.1 years, mean HbA1c 8.89 ± 1.4 % mean BMI 21.19 ± 3.4 kg/m² and mean basal insulin 36% of total insulin dose. Subjects were allocated to one of two groups regarding on basal rate reduction 90 minutes prior to physical activity (group A - 50% and group B - 0% of basal dose). Patients performed 45 minutes exercise on the cycle ergometer during two subsequent days. Primary outcome was the number of hypoglycaemic episodes, defined as blood glucose level below 70 mg/dl. Blood glucose concentrations were recorded with a continuous glucose monitoring system and with multiple blood glucose self-tests for 90 minutes prior to the study and 20, 45, 105 minutes after the beginning of the study. Other data were collected such as: height, weight, glycated haemoglobin, daily insulin dose.

Results: During the 45 minutes of exercise more hypoglycaemia episodes were noted in the group A (16%) than in the group B (4%), the difference was close to statistical significance $p=0.051$. Most hypoglycaemia episodes were noted in the 20th min. of the study. There was no statistically significant difference in blood glucose levels between both groups in each time-interval. Mean glycaemia in the group A vs. group B: at the start was 118 vs 108 ($p=0.148$); 20 min 127 vs 106 ($p=0.012$); 45 min 123 vs 116 ($p=0.115$); 60 min. after finishing the study 145 vs 129 ($p=0.144$). There was no between groups difference in the number of subjects with hyperglycaemia defined as blood glucose >160mg/dl 60 min. after finishing the study, OR 0.5 95%CI [0.2 to 1.2]; $p=0.186$. We did not found any correlation between basal insulin calculated as percentage of total insulin dose and blood glucose level during the study.

Conclusion: During prolonged exercise basal insulin dose reduction by 50% compared to the total cessation of insulin infusion was associated with a 4 fold higher risk of hypoglycemia. Therefore we recommend more than 50% reduction in basal rate during prolonged exercise. The degree of basal insulin reduction should be individually determined for each child.

PS 041 Exercise physiology II

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An acute bout of exercise is not able to lower liver fat content in overweight middle-aged men

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Background and aims: Elevated hepatic lipid content (IntraHepatic Lipid, IHL) markedly increases the risk of metabolic complications, including insulin resistance and cardiovascular events. Although prolonged exercise training has been shown to lower IHL, it is unknown if acute exercise has the same effect. Next to IHL, hepatic ATP content may be related to insulin resistance and IHL. Therefore, we aimed to investigate if acute exercise leads to changes in IHL and if these changes are accompanied by changes in hepatic ATP content.

Materials and methods: IHL content was determined by proton magnetic resonance spectroscopy (MRS) at baseline, directly after 2h of exercise (50 % of the maximal output) and again 4h post-exercise and expressed as percentage of the water resonance. Hepatic ATP content was measured by phosphorus MRS at baseline and 4h post-exercise in 8 subjects and is given relative to the total phosphorus signal. Blood was sampled and substrate oxidation was measured by indirect calorimetry. Since exercise leads to an increase in plasma FFA that may affect liver fat content, subjects performed the study not only in the fasted condition, but also while ingesting glucose.

Results: Twenty-one overweight middle-aged men with a wide range of IHL, including nonalcoholic fatty liver disease (age 54.8±7.2 years, BMI 29.7±2.2 kg/m²) participated in this study. IHL was unchanged directly after exercise (8.27±1.80 to 8.34±1.85 %). Similar effects were observed when exercising with glucose supplementation (IHL: 8.26±1.88 to 8.40±1.89 %). 4h after exercise, IHL was elevated compared to baseline in the fasted condition (from 8.3±1.8 to 8.7±1.8 %; $p=0.010$), while it did not change when glucose was supplemented (from 8.3±1.9 to 8.3±1.9 %; $p=0.789$). Hepatic ATP content tended to decrease post-recovery in the fasted condition compared to baseline values (0.164±0.009 vs. 0.146±0.009 γ -ATP/total phosphorus; $p=0.086$), whereas it did not change when glucose supplementation was given (0.168±0.008 vs. 0.163±0.005 γ -ATP/total phosphorus; $p=0.582$). Plasma FFA concentrations increased with time during exercise and recovery in the fasted condition ($p<0.0001$), but this effect was blunted in the glucose-fed condition. Respiratory quotient was significantly higher at all time points, except for baseline, in the glucose-fed state compared to fasted ($p<0.01$). Plasma glucose concentrations were comparable at baseline in both conditions (GLU: 5.26±0.11 vs. FASTED: 5.23±0.11 mmol/l), but increased after 2h of exercise and post-recovery in the glucose-supplemented state compared to the fasted condition (7.12±0.31 vs. 5.01±0.12 mmol/l, 6.51±0.26 vs. 4.49±0.11 mmol/l; $p<0.0001$). Moreover, plasma glucose increased with time during exercise and recovery in the glucose-supplemented condition ($p<0.0001$).

Conclusion: This data shows that an acute bout of exercise is not able to lower IHL, neither in a fasted nor in a glucose-supplemented condition, in overweight middle-aged men with a wide range of liver fat content. These results are in contrast to previous results on prolonged exercise training and demonstrate that the long term effects of training cannot simply be explained by an additive effect of acute exercise bouts.

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Carbohydrate doses before a 10 km competition in type 1 diabetes athletes

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Background and aims: Clinical guidelines recommend increasing the intake of carbohydrates (CH) to maintain glycaemic levels and avoid hypoglycaemia during exercise in type 1 diabetes (T1D) patients. Previous data indicate that the amount of CH recommended is higher than those actually consumed

by patients in competitions. The aim of this study was to identify the real consumption of CH during a 10Km competitive run and to compare these quantities with those recommended by current guidelines.

Materials and methods: Athletes with T1D, older than 18 years and treated with multiple insulin doses or an insulin pump participated in two different protocols. Protocol 1: Observational study including 31 athletes with T1D, collecting data of dietary records of CH intake before, during and after a 10Km competitive run. Data obtained were compared with those of a control group of 127 athletes without diabetes. Protocol 2: Single blind randomized trial in athletes with T1D, testing two different doses of pre-exercise CH supplements: a recommended dose of 0.7g CH/body weight ($n=10$) and a real dose (obtained after analyses from protocol 1) of 0.35g CH/body weight ($n=8$). This supplement was taken in the form of an isotonic beverage with a concentration of 6.5g of sucrose per 100ml. Capillary blood glucose was self-determined in all cases, before taking the supplement and at the end of competition.

Results: In the observational study, baseline characteristics were similar in both groups of individuals (table 1). Athletes with T1D consumed a lower quantity of CH at breakfast on a typical day and during the meal <1 hour after the competition. We did not find significant differences between groups in CH intake at breakfast or in the supplement before the competition. In T1D athletes, this supplement prior to the competition contains 25.0±11.3g of CH (0.33g CH/body weight). In the randomized study, athletes consuming the dose of 0.7g CH/body weight increased glycaemic levels significantly, from 144.3±88.6mg/dl starting to 211.1±100.1mg/dl ($p<0.05$) finishing the competition, while athletes taking 0.35g CH/body weight maintained glycaemic levels, from 197.5 ±38.9mg/dl before to 162.5±56.8mg/dl ($p=0.5$) after the competition.

Conclusion: On competition day, athletes with T1D seem to increase CH intake prior to the competition, both at breakfast and just before exercise, consuming similar amounts to those athletes without diabetes. In both cases, athletes with and without diabetes consume quantities of CH smaller than those recommended. A pre-exercise supplement with moderate-carbohydrate content (0.35g CH/body weight) appears to induce a more stable glycaemic response in comparison with a supplement with high-carbohydrate content in this group of athletes.

	T1D (n=31)	Control (n=127)	p value
Baseline Characteristics			
Gender (Male/Female)	20/11	87/40	0.7
Age (years)	36.1±10.0	40.2±9.0	0.2
Weight (kg)	74.8±13.2	71.8±11.4	0.2
BMI (kg/m ²)	24.7±4.6	23.7±2.6	0.2
Training time (hours·week ⁻¹)	237.9±150.2	291.4±273.7	0.5
Physical activity levels (MET·min·week ⁻¹)	52.2±29.9	58.2±51.06	0.7
Diabetes evolution (years)	9.9±8.9	--	--
Glycated Haemoglobin (%)	7.2±0.8	--	--
Insulin requirements (units·kg body weight ⁻¹ ·day ⁻¹)	0.5±0.2	--	--
Long-acting insulin (units/day)	22.1±8.9	--	--
Short-acting insulin (units/day)	16.3±9.5	--	--
Glycaemic tests (weekly frequency)	32.3±16.7	--	--
Carbohydrate consumption			
CH at breakfast on a typical day (g)	35.6±17.1	47.7±24.1	<0.05
CH at breakfast on competition day (g)	43.4±19.0	47.3±26.2	0.4
CH <1hour before competition (g)	25.0±11.3	19.1±9.3	0.2
CH <1hour after competition (g)	24.9±12.2	34.4±16.6	<0.05

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Exercise induces an anti-inflammatory response and positive effects on the metabolic status in a Non-Obese Diabetic (NOD) mouse model

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Background and aims: The Non-Obese Diabetic (NOD) mouse represents a well-established experimental model analogous to human type 1 diabetes mellitus (T1DM) as it is characterized by a progressive autoimmune destruction of pancreatic β -cells. NOD mouse might provide a useful model for the study of exercise in T1DM. Experiments were designed to investigate the impact of moderate-intensity training on T1DM immune modulation.

Materials and methods: Under a chronic exercise regime, NOD mice ($n=20$; ♀; 8-wk old) were trained on a treadmill for 12 weeks (12m/min for 30min,

5d/wk) while same number, age-matched, control animals were sedentary. Prior and upon completion of the training period, fed plasma glucose and immunological soluble factors were monitored.

Results: After 12 weeks of training, control mice were all diabetic ($n=5$) whereas only 2 out of 5 mice on-exercise became diabetic. An exercise-induced weight loss (-9% , $p<0.05$) was detected in the trained mice with respect to the controls. As to the cytokines results, while control mice did not show any changes in the inflammatory factors at end of the training program with respect to baseline, we observed a slight but significant decrease of MIP-1 β (-1%), IFN- γ (-2%), IL-10 (-2%), IL-2 (-1%) IL-13 (-2%), GM-CSF (-2%), and a substantial increase in G-CSF ($+27\%$) in the trained animals compared to their pre-training values ($p<0.05$). Preliminary data from a morphometric analysis of pancreata indicated the presence of larger infiltrates along with increased α -cells areas in the control mice.

Conclusion: In summary, moderate-intensity exercise induced an anti-inflammatory effect in the trained mice paralleled by a greater amount of infiltrates in the disease-related deterioration of the control mice. We speculate that exercise may exert a positive immunomodulation of systemic functions with respect to both T1DM and inflammation.

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Sesamin improves lowered exercise capacity and mitochondrial dysfunction in the skeletal muscle from high fat diet-induced diabetic mice

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Background and aims: We previously reported that high fat diet (HFD)-induced diabetic mice had the reduced exercise capacity and impaired mitochondrial function in the skeletal muscle, which was due to NAD(P)H oxidase-induced oxidative stress. Sesamin is a natural ingredient included in sesame (*Sesamum indicum*) which is a traditional herbal medicine, and has multiple biological functions such as antioxidant activity. We hypothesized that sesamin could ameliorate the reduced exercise capacity in diabetic mice.

Materials and methods: C57BL/6J mice were fed on normal diet (ND) or HFD, and each group of mice was treated with or without sesamin (0.2%) in the diet for 8 weeks. Exercise capacity and mitochondrial function in the skeletal muscle were evaluated.

Results: HFD significantly increased adipose tissue weight and plasma glucose compared to ND, and sesamin did not affect these parameters in either group ($p>0.05$). HFD significantly increased body weight and plasma insulin compared to ND (body weight; 43 ± 1 vs 29 ± 1 g, insulin; 2.0 ± 0.3 vs 0.5 ± 0.1 ng/mL, $p<0.05$), and these gains were ameliorated in HFD+sesamin (body weight; 37 ± 1 g, insulin; 0.8 ± 0.2 ng/mL, $p<0.05$). The work and peak oxygen uptake determined by treadmill tests were significantly reduced in HFD compared to ND (work; 14 ± 1 vs 28 ± 1 J, peak VO_2 ; 109 ± 4 vs 152 ± 2 mL/kg/min, $p<0.05$), and these reductions were ameliorated in HFD+sesamin (work; 20 ± 1 J, peak VO_2 ; 129 ± 2 mL/kg/min, $p<0.05$). Citrate synthase (CS) activity was significantly decreased, and NAD(P)H oxidase activity and superoxide production by lucigenin chemiluminescence were significantly increased in isolated skeletal muscle from HFD compared to ND (CS activity; 31 ± 3 vs 108 ± 6 nM/min/mg protein, NAD(P)H oxidase activity; 19 ± 1 vs 5 ± 1 RLU/min/mg protein, superoxide anion; 55 ± 9 vs 15 ± 2 RLU/min/mg protein, $p<0.05$), which were also ameliorated by sesamin (CS activity; 70 ± 8 nM/min/mg protein, NAD(P)H oxidase activity; 8 ± 1 RLU/min/mg protein, superoxide anion; 18 ± 2 RLU/min/mg protein, $p<0.05$). Additionally, *in vitro* data showed that sesamin and its noma-catechol metabolite significantly suppressed angiotensin II-induced NAD(P)H oxidase activity in C2C12 myotubes ($p<0.05$).

Conclusion: Sesamin ameliorated the reduced aerobic exercise capacity in HFD-induced diabetic mice via the improvement of impaired mitochondrial function and attenuation of oxidative stress in the skeletal muscle.

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High intensity intermittent exercise reverses abnormal cardiac function in people with type 2 diabetes: an MRI/S study

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Background and aims: Cardiovascular disease is the leading cause of morbidity and mortality in Type 2 diabetes. Despite the clear individual and societal burden of cardiovascular disease in Type 2 diabetes, evidence based therapies to improve cardiac function are limited. High intensity intermittent exercise is a time efficient strategy for cardiovascular training, but its direct effects on cardiac structure and function in people with Type 2 diabetes are unknown. This study investigated high intensity intermittent exercise as a potential therapeutic tool to moderate cardiac risk in this patient group.

Materials and methods: Twenty-three patients with Type 2 diabetes (age 60 ± 9 years, BMI 31 ± 5 kg/m², HbA_{1c} 55 ± 8 mmol/mol) were randomised to 12 weeks of high intensity intermittent exercise (treatment, $n=12$) or standard care (controls, $n=11$). High intensity intermittent exercise consisted of 5 x 2min intervals at $>80\%$ heart rate max using cycle ergometry three times a week (guided by a podcast). Each week the duration of each exercise bout was increased. Cardiac structure, function and energetics were measured respectively by 3.0 T magnetic resonance imaging, 2 dimensional tagging and phosphorus-31 spectroscopy. Liver fat was determined by ¹H-magnetic resonance spectroscopy.

Results: Compared to controls, high intensity intermittent exercise improved cardiac structure (Left ventricular Mass- 104 ± 17 to 116 ± 20 vs. 107 ± 25 to 105 ± 25 g, $p<0.05$; End-diastolic blood volume- 118 ± 30 to 126 ± 30 vs. 129 ± 28 to 122 ± 28 ml, $p<0.05$) and cardiac systolic function (stroke volume- 76 ± 16 to 87 ± 19 vs. 79 ± 14 to 75 ± 15 ml, $p<0.05$; Left ventricular ejection fraction- 65 ± 8 to 70 ± 6 vs. 64 ± 11 to $63\pm 10\%$, $p<0.05$). Early diastolic filling rates increased (241 ± 84 to 299 ± 89 vs. 250 ± 44 to 251 ± 47 ml/s, $p<0.05$) and peak torsion decreased (8.1 ± 1.8 to 6.9 ± 1.6 vs. 7.1 ± 2.2 to $7.6\pm 1.9^\circ$, $p=0.05$) in the treatment group compared to controls. There was no effect on cardiac metabolism determined by magnetic resonance phosphorus-31 spectroscopy. High intensity intermittent exercise also delivered benefits to glucose control (HbA_{1c}- 55 ± 11 to 51 ± 10 vs. 55 ± 6 to 57 ± 8 mmol/mol; $p<0.05$) and liver fat (7 ± 7 to 4 ± 4 vs. 7 ± 7 to $8\pm 7\%$; $p<0.05$).

Conclusion: High intensity intermittent exercise has positive effects on cardiac structure and function alongside benefits to glucose control and liver fat. Clinical care teams should consider high intensity intermittent exercise as a therapeutic strategy to moderate cardiac dysfunction in people with Type 2 diabetes.

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Physical activity patterns in a type 2 diabetes population. Relationship with cardiovascular risk factors, diabetes complications and comorbidities

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Background and aims: The aim of our study is to analyze the physical activity (PA) pattern and risk factors associated with sedentary T2DM population.

Materials and methods: Cross-sectional multicenter random study in primary care. Physical Activity (PA) was assessed by Short International PA Questionnaire with 4 categories: Low or Sedentary (<600 MET/week), Moderate-medium (600-1499), Moderate-High (1500-2999) and High (≥ 3000). We registered sociodemographic, anthropometric data and cardiovascular (CV) risk factors, disease and risk scores. Yesavage and Mediterranean Diet test and DM complications and comorbidities were also compiled. Analytical data: HbA_{1c}, lipid, renal profile and uricaemia. Bivariate and multivariate logistic regression (PA low versus moderate to high) analyses were performed.

Results: Of 447 patients, 57.9% were men. Mean age: 67.9 ± 10.4 ; mean HbA_{1c}: $7.1\pm 1.2\%$; 36.6% had overweight and 50.7% obesity I (BMI 30-34); 28.6% and 20.8% had CV and Chronic Renal Disease (CRD), respectively. PA pattern was: 24.4% low, 28.3% moderate-medium, 21.7% moderate-high

and 25.6% high. Low PA was more frequent in: women, ≥ 75 years, smokers, hypertensive, patients with higher BMI and waist risk circumference, higher Stroke scores, CRD, depression, and elevated lipid profile. Independent risk factors associated with low PA in the multivariate analysis were (Odds ratios, CI 95%): Hypertension (3.1, 1.5–6.2), CRD (1.8, 1.04–3.2), depression (2.7, 1.2–5.9), longer time of T2DM onset (1.04, 1.0–1.09), higher levels of triglycerides (1.01, 1.0–1.01) and ldl (1.01, 1.0–1.02). By contrast, have a good control of glycaemia (0.5, 0.3–0.8), of blood pressure (0.4, 0.3–0.8) and of triglycerides (0.4, 0.2–1.0) were protective factors for sedentary. Female had a higher risk for sedentary in the limit of the significance (OR: 1.5; CI95%: 0.9–2.5).

Conclusion: 24% of our T2DM population was sedentary. PA improves glycaemia control, lipid profile, CV risk factors and CRD, all of them very important to prevention of diabetes morbidity. Inactivity is also related with a risk of depression, probably associated to the quality of life.

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Lowered muscle endurance in association with neuropathy in patients with type 2 diabetes

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Background and aims: Type 2 diabetes is characterized by a reduced sensitivity of the tissues to insulin (insulin resistance), and a reduced formation of insulin by the pancreas. This is often accompanied by damage to blood vessels and nerves (neuropathy). The treatment of diabetes includes medication, nutritional advice and exercise therapy. Strength training has a positive effect on insulin resistance and muscle function. What kind of strength training is most optimal, remains unknown. To investigate this, first of all, it should be examined to what extent different types of strength (maximal-, explosive-, endurance,) are influenced by the severity of diabetes, taking into account additional conditions, such as peripheral neuropathy. Since the negative influence of diabetes and neuropathy on maximal strength has already been investigated, the purpose of this study was to investigate the association between neuropathy and arm/leg muscle endurance.

Materials and methods: Data from an isokinetic dynamometer, a clinical neurological examination + quantitative sensory examination and nerve conduction studies were obtained in 35 type 2 diabetic men, with (n=24) and without neuropathy (n=11). Seven patients had small fiber sensory neuropathy, 17 had sensorimotor neuropathy. All men performed 25 elbow- and 30 knee maximal concentric contractions on an isokinetic dynamometer at a given angular velocity of 180°/sec. Elbow- and knee flexor fatigue was measured with the fatigue index, calculated as the ratio of the average peak torque of the last third to the average peak torque of the first third. A neuropathy rank sum score (NRSS) was calculated for each patient to quantify the peripheral nerve function, adding the rank scores for the Michigan Neuropathy Screening Instrument (MNSI) questionnaire, the neuropathy disability score (NDS), the vibration perception thresholds (VPT's), the motor and sensory nerve conduction velocities (MNCV's and SNCV's), as well as the amplitudes of the compound muscle - and sensory nerve action potentials (CMAP's and SNAP's). The VPT's were ranked on the basis of the sum of the two percentiles obtained at right and left great toe. The EMG-parameters (MNCV & CMAP of peroneal, tibial and ulnar nerve, SNCV & SNAP of radial and sural nerve) were ranked for all nerves according to the calculated z-score. In order to calculate these z-scores, MNCV + CMAP and SNCV + SNAP were measured in 16 healthy subjects in an age-matched comparison group. Finally, the mean sum score for the conduction studies was used for the total rank sum score. If no action potential could be obtained, the highest rank score was given. A high NRSS thus reflected severe nerve dysfunction. To estimate the correlations between the neuropathy rank sum score and the fatigue indices, pearson correlation analysis was used.

Results: A significant negative correlation was indicated between the isokinetic fatigue index of knee flexors and the NRSS ($r = -0.356$, $p = 0.036$). For elbow flexors there was also a tendency towards a negative correlation with the NRSS ($r = -0.318$, $p = 0.062$).

Conclusion: This finding suggests that not only maximal strength, but also endurance in diabetic people with neuropathy is worse than in diabetics without neuropathy.

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Characteristics of patients with type 2 diabetes who achieved better glycaemic control through resistance training: a meta-analysis

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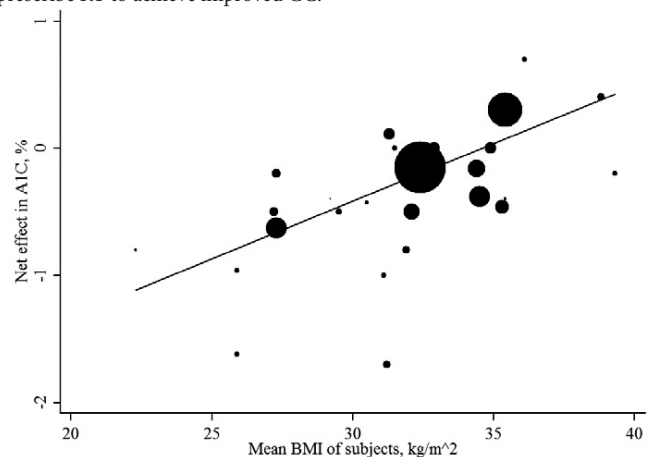
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Background and aims: Recently, an increasing number of studies have suggested that resistance training (RT) has been effective in improving glycaemic control (GC) in patients with type 2 diabetes (T2D). Our aim of this meta-analysis is to determine the antihyperglycemic effect of RT and analyze characteristics of patients for whom RT could be especially indicated for improvement in GC.

Materials and methods: Electronic-based literature search was conducted for intervention studies examining the metabolic effect of RT in T2D patients using MEDLINE and EMBASE. Studies were included if 1) both an RT-exercise group and non-RT control group were examined and 2) the net change in hemoglobin A_{1c} (A1C) (i.e., difference in mean change in A1C from before to after the study between RT and non-RT groups) and its corresponding standard error as a study weight could be estimated. We included studies that described other interventions such as aerobic training if those other interventions were similar between the RT and non-RT groups.

Results: Twenty-seven studies were eligible. Net change in A1C (95% confidence interval [CI]) was -0.3 [-0.46 – -0.14]. A significantly larger reduction in A1C was observed in studies of subjects with the following characteristics: 1) short duration of T2D (≥ 6 years vs. < 6 years, $P = 0.03$), 2) women-dominant ($< 50\%$ vs. $\geq 50\%$ proportion of men, $P = 0.006$) and 3) high A1C values at baseline ($\geq 7.5\%$ vs. $< 7.5\%$, $P = 0.006$). There was a linear relationship between baseline body mass index and the net effect on A1C (R -squared = 0.42 , $P < 0.001$), predicting that a 1 kg/m^2 leaner subject would achieve a 0.09% larger reduction in A1C after RT (Figure). The training protocol such as intensity, interval between sets, frequency, and intervention periods did not significantly modify study results.

Conclusion: This meta-analysis produced useful suggestions regarding the characteristics of patients with T2D for whom clinicians should especially prescribe RT to achieve improved GC.



Relationship between mean body mass index (BMI) and the effect of resistance training (RT) on glycemic control. Net change in hemoglobin A1C (A1C) (i.e., change in A1C in RT group minus that in non-RT group from before to after the intervention study) was plotted against mean BMI in study subjects. The area of circles is proportional to study weight (i.e., inverse of square of standard error of the net effect in A1C)

PS 042 Consequences of hypoglycaemia

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Severe hypoglycaemia requiring medical assistance in patients with diabetes is associated with simultaneous prolongation of QTc interval

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Background and aims: It has been shown that hypoglycemia induces multiple pre-arrhythmic changes, mainly increase pre-existent QT prolongation, produce intracellular Ca^{2+} overload, and decrease serum K^+ . As a consequence, hypoglycemia is considered a state that may lead to sudden death, as a result of cardiac arrhythmia. Few data, however, directly link episodes of severe hypoglycemia with pre-arrhythmic conditions, such as QT prolongation. Aim of the present study is to explore the association between severe hypoglycemic events requiring medical assistance and QT interval, in patients with diabetes.

Materials and methods: Eight hospitals (nine clinics), in five cities of Greece, participated in a 16-month prospective survey of documented iatrogenic hypoglycemia at the emergency departments (ED). According to the protocol, a 12-lead ECG was obtained simultaneously or immediately after the management of hypoglycemia and no later than 30 minutes after the administration of glucose. The ECGs obtained from hypoglycemic patients were compared to those from a control group consisted of patients with diabetes, matched for age and gender, visiting the outpatient diabetes clinics of the same hospitals, during the same time period. QT and RR intervals were measured blindly by three independent cardiologists. QTc was calculated according to the Bazett formula: $\text{QTc} = \text{QT interval} / \sqrt{\text{R-R interval}}$. QTc measurements of ≥ 440 msec were considered abnormally prolonged and those of ≥ 500 msec as highly prolonged. Patients receiving medications possibly affecting the QTc interval and those with hypokalaemia (serum potassium < 3.5 mEq/l) were excluded from the analysis.

Results: During a median follow-up of 16 months, 295 episodes of iatrogenic hypoglycemia in 294 patients with diabetes were identified and 223 ECGs were obtained according to the pre-specified criteria. However, 46 ECGs were excluded from the analysis, due to the presence of the above mentioned criteria. Finally, 177 ECGs from hypoglycemic patients were analyzed (mean age 72.7 ± 15.7 years, 48.6% women, 9% with type 1 diabetes) and compared to 91 age and gender matched controls. Mean QTc interval was significantly prolonged in patients compared to controls (440.4 ± 45.1 msec vs. 413.9 ± 32.5 msec, $p < 0.001$). In addition, a significantly higher proportion of hypoglycemic patients had an abnormally prolonged QTc (≥ 440 msec) compared to controls (49.7% vs. 24.2%, $p < 0.001$), while 14 hypoglycemic patients (7.9%) had a highly prolonged QTc (≥ 500 msec) compared to none in controls.

Conclusion: In patients with diabetes, severe iatrogenic hypoglycemia requiring medical assistance is associated with a both statistically and clinically significant prolongation of QTc interval.

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Facial skin temperature measured by infrared thermography during hypoglycaemia in patients with longstanding type 1 diabetes

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Background and aims: Hypoglycaemia is associated with increased activity in the autonomic nervous system resulting in increased sweat production and changes in peripheral blood flow. Since peripheral blood flow influences overlying skin temperature (Ts), hypoglycaemia may cause changes in skin temperature. The aim of this study was to investigate changes in Ts during induced hypoglycaemia in patients with long standing type 1 diabetes. We hypothesized that hypoglycaemia results in decreased Ts.

Materials and methods: Twenty-four patients with type 1 diabetes participated in the study (14 men, age 54 ± 12 years (mean \pm SD), duration of diabetes 28 ± 11 years, HbA1c 8.0 ± 1.0 %). Fourteen patients had hypoglycaemia unawareness and 10 patients had normal hypoglycaemia awareness. The patients were studied during euglycaemia (5.4 ± 0.6 mmol/L) and hypoglycaemia (2.4 ± 0.2 mmol/L) by a hyperinsulinaemic glucose clamp technique. During each phase of one hour, Ts was measured by infrared thermographic imaging in two pre-defined facial areas of approximately 2 cm² size on the forehead just above the bony nose and on the tip of the nose. Autonomic symptoms of hypoglycaemia were recorded and venous blood was sampled.

Results: During hypoglycaemia Ts decreased on the forehead ($\Delta\text{Ts} = -0.86 \pm 0.99^\circ\text{C}$, $p=0.001$) and on the nose ($\Delta\text{Ts} = -1.75 \pm 2.1^\circ\text{C}$, $p=0.002$). There was a difference in Ts changes on the forehead between the two groups ($\Delta\text{Ts} = -1.4 \pm 0.29^\circ\text{C}$ in aware patients vs $\Delta\text{Ts} = -0.4 \pm 0.23^\circ\text{C}$ in unaware patients, $p=0.01$) (Figure). Epinephrine concentrations increased during hypoglycaemia compared to euglycaemia (0.49 ± 0.42 ng/mL vs 0.07 ± 0.05 ng/mL, $p < 0.001$) with no significant difference in rise of epinephrine between aware and unaware patients (0.54 ± 0.52 ng/mL vs 0.34 ± 0.31 ng/mL, $p=0.19$). Autonomic symptom scores increased during hypoglycaemia (10 ± 6 points vs 5.5 ± 1.8 points, $p=0.001$). The aware group reported 2.5 times higher symptom scores during hypoglycaemia than the unaware group (15 ± 5.9 points vs 6.5 ± 2.6 points, $p < 0.01$).

Conclusion: Hypoglycaemia-associated changes in facial skin temperature can be quantified by infrared thermography in patients with type 1 diabetes. Patients with normal awareness had a larger Ts decrease on the forehead compared to patients with hypoglycaemia unawareness and more autonomic symptoms during hypoglycaemia despite no significant difference in blood epinephrine concentrations between the two groups.

Skin temperature on the forehead during hypoglycaemia

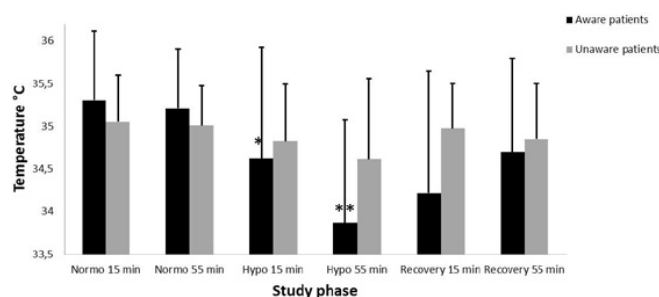


Figure: Temperature changes on the forehead during hypoglycaemia. Measurements were performed 15 and 55 min into the normoglycaemic, hypoglycaemic and recovery phases. * $p=0.03$, ** $p < 0.001$ when compared to normoglycaemia.

Clinical Trial Registration Number: H-1-2011-024

Supported by: Nordsjællands Hospital Research Foundation

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Cardiac autonomic regulation during acute experimental hypoglycaemia in type 2 diabetes

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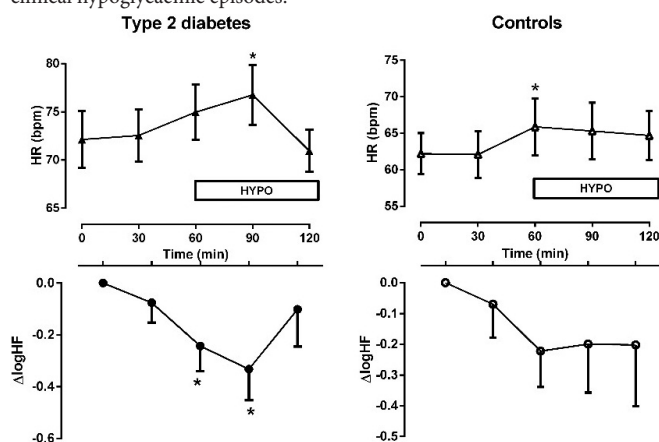
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Background and aims: Hypoglycaemia is associated with increased cardiovascular mortality in trials of intensive therapy in type 2 diabetes. In recent clinical studies involving type 2 diabetes patients, we reported a higher incidence of ventricular ectopics and bradyarrhythmias during spontaneous prolonged hypoglycaemia, which we attributed to differential changes in autonomic tone. In this study our aim was to examine changes in cardiac autonomic function in more detail during sustained experimental hypoglycaemia.

Materials and methods: Twelve type 2 diabetes subjects with normal cardiac autonomic function tests and eleven age, BMI-matched nondiabetic controls underwent paired hyperinsulinaemic clamps separated by 4 weeks. In the euglycaemic arm, glucose was maintained at 6.0 mmol/L for 2h while during hypoglycaemia, glucose was lowered to 2.5 mmol/L over 1 hour and maintained for a further hour. Heart rate (HR), blood pressure and heart rate variability (HRV) were assessed at 30 min intervals. Spectral power of HRV was calculated within low frequency (LF: 0.04-0.15 Hz) and high frequency (HF: 0.15-0.4 Hz) intervals.

Results: In the diabetic group, HR increased maximally from 72 ± 10 to 77 ± 10 bpm ($p=0.04$) after 30 minutes of hypoglycaemia (T90), but fell to 71 ± 8 bpm despite maintained hypoglycaemia (T120) (see Fig). High frequency HRV decreased up to T90 ($\log HF$ 1.89 ± 0.61 vs 1.61 ± 0.63 , $p=0.02$), indicating acute vagal withdrawal, but returned towards baseline at T120. There was a rise in normalised low frequency power indicating increased sympathetic contribution. In the control group, the maximal increase in HR occurred earlier at T60 during hypoglycaemia (from 62 ± 9 to 66 ± 13 bpm, $p=0.04$) but HR remained elevated at 65 ± 11 bpm after hypoglycaemia for 1 hour. HF power decreased from baseline ($\log HF$ 2.36 ± 0.44 to 2.14 ± 0.59 , $p=0.05$) and remained unchanged despite continued hypoglycaemia, indicating inhibition of vagal tone throughout. No significant changes in HRV occurred in either group during euglycaemic clamps.

Conclusion: Cardiac autonomic regulation during hypoglycaemia appears time-dependent and differs between patients with type 2 diabetes and non-diabetic individuals. In type 2 diabetes, the initial heart rate increment to hypoglycaemia is delayed. Following early vagal withdrawal, there is reactivation of vagal activity during more sustained hypoglycaemia which did not occur in nondiabetic individuals. These mechanisms may relate to autonomic dysfunction and could contribute to tachy- and bradyarrhythmias during clinical hypoglycaemic episodes.



Clinical Trial Registration Number: DRN686

Supported by: NIHR

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Survey of outpatients with type 2 diabetes among older adults in Japan

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Background and aims: The number of older adults with Type II diabetes (T2DM) is continuously increasing in Japan, and care needs to be provided according to the individual needs of these patients, which require more urgent attention in Japan than in the West. Despite this situation, few studies have investigated the prevalence of hypoglycemia among older T2DM in Japan. This study describes the results of a survey conducted by the Japanese Physicians Association (JPA) to elucidate the prevalence of hypoglycemia among older T2DM in Japan. The survey collected data on patient knowledge about hypoglycemic symptoms as well as their clinical background.

Materials and methods: A total of 15,892 older adults aged ≥ 65 years (8319 men and 7516 women) who were undergoing regular outpatient treatment for their diabetic conditions were included in this study. The survey included questions pertaining to 28 items concerning hypoglycemic symptoms and age-related syndromes experienced in the last month. The JPA also collected patients' medical records from their doctors.

Results: The average age of patients was 74.2 years (men, 73.6 years; women, 75.0 years), the mean disease duration was 12.8 years (men, 13.1 years; women, 12.5 years), and the mean body mass index (BMI) was 24.1 (men, 24.0; women, 24.2). Medications used for diabetes treatment, including concomitant prescriptions, were as follows: DPP-4 inhibitors, 53.3%; SU, 39.4%; α -GI, 23.4%; metformin, 22.8%; pioglitazone, 14.0%; glinide, 4.4% and insulin, 17%. Although the incidence of hypoglycemic symptoms as estimated by the doctors on the basis of their diagnoses in the previous month was 7.8%, the actual incidence indicated by the patient responses was 10.4%. The responses indicated that the incidence of hypoglycemia among patients treated with either SU or insulin was 20.9%, whereas that among patients treated

without neither drugs was lower at 2.5%. Tiredness/feeling languid (32.8%), loss of balance/dizziness (32.4%), cold sweats (30.4%), and eye blink (24.8%) were the most frequently experienced hypoglycemic symptoms. Among the patients who used SU or insulin, 80.1% were aware of their hypoglycemic symptoms and 64.4% knew how to treat these symptoms. However, only 38.1% patients who were aware of their hypoglycemia always carried some form of glucose with them.

Conclusion: The results of our survey indicated that the symptoms of hypoglycemia were atypical and vague in older T2DM patients, and the actual incidence of hypoglycemia among the patients was considerably higher than that estimated by the doctors on the basis of their diagnoses. Although there was satisfactory awareness of hypoglycemia among the older adults, two-thirds of them did not always carry some form of glucose with them. Therefore, more careful treatment of T2DM and more positive measures to promote the awareness of hypoglycemia are required for older adults with T2DM.

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Impaired awareness of hypoglycaemia in insulin-treated patients with type 2 diabetes mellitus

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Background and aims: Severe hypoglycemia (SH) is associated with a higher risk of cardiovascular disease-related mortality and mortality. Impaired awareness of hypoglycemia (IAH) in insulin-treated patients with type 2 diabetes mellitus (T2DM) has been reported to be associated with the presence of SH. However, little is known regarding the actual states of hypoglycemia, including IAH, in Japan. The aim of the study was therefore to survey the states of hypoglycemia and patient-physician communication among insulin-treated patients with T2DM.

Materials and methods: A questionnaire survey on hypoglycemia and patient-physician communication was conducted in 331 patients with insulin-treated T2DM patients at 16 hospitals and clinics. Inclusion criteria were: 1) over 20 years, 2) T2DM, 3) insulin treatment, 4) patients regularly attended the surveyed hospitals and clinics. Exclusion criteria were: 1) children and young patients under 20 years old, and 2) type 1 diabetes mellitus. Clinical data such as the insulin regimen and diabetic complications were collected from their physicians. Hypoglycemia was defined as blood sugar ≤ 50 mg/dL (2.8 mmol/L) or symptoms of dizziness, blurry vision, confusion, and/or sweating that the patient was able to resolve without assistance. Similar symptoms that required external assistance were defined as SH. Favorable glycaemic control to prevent diabetic complications was defined as HbA1c $< 7\%$ according to "Kumamoto declaration 2013". Patient-physician communication regarding hypoglycemia and strategies to avoid it was based on the answer categories "never" and "seldom" as poor communication, and "sometimes" and "often" as favorable communication, in response to "how often do you talk about hypoglycemia and strategies to avoid it with your physician?".

Results: The rate of IAH was 22.1%. There were no significant differences in clinical characteristics, such as the age, diabetes duration, prevalence of hypoglycemia, insulin regimen, and diabetic complications, between patients with and without IAH. The rates of alcohol drinking and smoking in males were significantly higher than in females (22.5 vs. 1.4%; $p < 0.01$, and 30.9 vs. 12.2%; $P < 0.01$, respectively). A stepwise logistic regression analysis revealed that glycaemic control at an HbA1c level $< 7.0\%$ was a significant risk factor of IAH (odds ratio [OR]: 2.12; 95% confidence interval [CI]: 1.22–3.68; $P = 0.01$), along with a male sex (OR: 1.89; 95% CI: 1.06–3.38; $P = 0.03$) and a BMI level (OR: 0.89; 95% CI: 0.82–0.97; $P = 0.01$). Concerning talking about hypoglycemia with physicians, patients with IAH showed a similar frequency of talking with physicians as patients without IAH (76.1 vs. 71.4%; $P = 0.44$, respectively), while patients with SH communicated more frequently than those without SH (90.9 vs. 70.3%; $P = 0.01$, respectively).

Conclusion: The current survey found that IAH was not uncommon among insulin-treated patients with T2DM in Japan. Aging, a lower BMI, and lower HbA1c levels were significantly correlated with IAH. The patients with SH talked less about hypoglycemia with their physicians. To improve the actual states of hypoglycemia, physicians should be conscious of the factors associated with IAH, and should talk more about hypoglycemia to insulin-treated patients with T2DM in Japan.

Supported by: National Hospital Organization

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Hypoglycaemia unawareness in emerging adults transferred from a paediatric to an adult diabetes unitM.C. Careaga¹, M. Vidal¹, M. Jansa¹, C. Yoldi², R. Cardona², A. Gomez², M. Gimenez¹, E. Esmatjes¹, I. Conget¹;¹Diabetes Unit, Endocrinology and Nutrition Department, Hospital Clinic and University of Barcelona,²Pediatric Endocrinology, Hospital Sant Joan de Deu, Barcelona, Spain.

Background and aims: Repeated hypoglycemia and the syndrome of hypoglycemia unawareness (HU) are major barriers to achieving normoglycemia over a lifetime of using intensive insulin therapy and thus precludes euglycemia's long-term benefits. Type 1 Diabetes (T1D) management in young patients is troublesome specially when they are transferred to adult Diabetes Units. The aim of our study was to evaluate the impact of the presence of HU on the results of a transition therapeutic education program (TEP) specifically addressed to the transfer T1D patients from a pediatric to an adult diabetes Unit.

Materials and methods: We included 56 patients with T1D (age 18.0 ± 0.3 years, 26 girls, disease duration 8.0 ± 4.0 years, HbA1c $8.0 \pm 1.2\%$) consecutively transferred (2009–2011). The TEP included a co-ordinated transfer between the Units, individual visits and group sessions. At baseline, we registered data on BMI, insulin dose, HbA1c and the frequency of hypoglycemia. For evaluation of the quality of life (QoL) and knowledge in T1D management, self-report questionnaires were assessed. Mother tongue (spanish and catalan versions) validated Clarke's test was used to evaluate HU. After 12-months, all subjects were re-evaluated.

Results: Nine out of 56 patients had HU (16%). Age, gender, duration of disease, type of treatment, HbA1c, the different aspects of QoL and the degree of knowledge of the disease were not different in those subjects affected by HU in comparison to the unaffected group. Only the number of severe hypoglycemia episodes was significantly different between groups (0.33 ± 0.50 vs. 0.09 ± 0.28 , episodes-subject-year with and without HU respectively, $p = 0.13$). 5 non severe episodes/week was close to significantly different (66 vs 34% , $p = 0.13$). After 12 months, in the group with HU, 4 patients improved awareness, there was a reduction in the number of non severe hypoglycemia episodes/ week, but this was not the case of severe episodes. The HbA1c was not significantly different between the two groups (8.2 ± 1.1 vs. $7.6 \pm 0.9\%$ with and without HU, respectively).

Conclusion: The presence of HU is far from uncommon when emerging adults with T1D are transferred from a pediatric to an adult diabetes unit. While the use of an specific TEP improves metabolic prognosis in the mid-term, the reduction of hypoglycemia episodes, specially severe, remains an elusive issue

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Timing of hypoglycaemia is associated with increased mortality and length of stay among patients with diabetes admitted to internal medicine departmentsJ. Wainstein¹, M. Boaz^{2,3}, E. Leibovitz^{4,5};¹Diabetes clinic, Wolfson Medical Center, ²Research and Epidemiology, Wolfson Medical Center, Holon, ³Epidemiology, Tel Aviv University,⁴Internal Medicine E, Wolfson Medical Center, Holon,⁵Sackler school of Medicine, Tel Aviv University, Israel.

Background and aims: Hypoglycemia among hospitalized patients with diabetes is associated with poor hospital prognosis. The aim of the study was to examine the effect of hypoglycemia timing on hospital prognosis

Materials and methods: In this retrospective analysis of electronic medical records, we included all 3941 patients with diabetes (Mean age 71.7 ± 12.9 years, 49.3% males) discharged from internal medicine departments during 2009. All glucose measurements were computerized using an institutional glucometer. Patients were categorized into 3 groups according to hypoglycemia timing: Group 1, no hypoglycemia, Group 2, hypoglycemia only upon admission and Group 3, hypoglycemia during hospital stay.

Results: Included in the analysis were 3413 (86.4%) patients with diabetes who had glucose measurements performed during hospitalization. A total of 157 patients (4.6%) had at least 1 hypoglycemia event during the hospital stay (42 patients upon admission and 115 patients during hospitalization). Patients in Group 3 had significantly increased in-hospital mortality and length of hospital stay (27.0% , 18.4 ± 24.7 days), compared to Groups 1 (3.4% mortality, 5.1 ± 8.8 hospital days) and Group 2 (4.7% mortality, 3.2 ± 3.3 hos-

pital days). Mortality rates were associated with the severity and number of hypoglycemia events. Patients in Group 3 had higher creatinine and lower albumin levels compared to Groups 1 and 2.

Conclusion: Hypoglycemia during hospitalization is associated with increased rates of in-hospital mortality and prolonged hospital stay among diabetic patients admitted to internal medicine departments. Poor hospital outcome was associated with timing of hypoglycemia, as well as the number and severity of the events.

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Baseline insulin deficiency is associated with increased mortality and severe hypoglycaemia in the ACCORD studyE.R. Seaquist¹, H. Chen², M. Miller², L. Chow¹;¹Medicine, University of Minnesota,²Biostatistics, Wake Forest School of Medicine, Winston Salem, USA.

Background and aims: Intensive glycemic control is associated with increased mortality, but the role of hypoglycemia remains unclear. Hypoglycemia is associated with insulin deficiency. Whether insulin deficiency and/or islet autoimmunity may affect mortality in patients with type 2 diabetes remains unknown. Using the ACCORD (Action to Control Cardiovascular Risk in Diabetes) cohort, we tested the hypothesis that baseline insulin deficiency and islet autoimmunity in type 2 diabetes would be associated with severe hypoglycemia and mortality.

Materials and methods: A nested case-control study (86 cases, 344 controls) was used. A participant who died during ACCORD with at least 1 episode of severe hypoglycemia, defined as hypoglycemia requiring assistance, was classified as a case. Participants who did not die during ACCORD and did not have severe hypoglycemia were classified as controls. Each case was matched to 4 controls [age (± 5 yrs), BMI (± 2.5), glycemic intervention arm, race]. Insulin deficiency was defined as fasting C-peptide < 0.45 nmol/L. Islet autoimmunity was determined by antibodies against glutamic acid decarboxylase (GAD), tyrosine phosphatase-related islet antigen 2 (IA-2A), insulin (IAA), and zinc transporter (Zn-T8). Conditional logistic regression was used.

Results: Death during ACCORD with at least 1 episode of severe hypoglycemia was associated with baseline insulin deficiency (OR 4.8, 95% CI 2.1–11.1, $p < 0.01$) and GAD antibodies (OR 2.3, 95% CI 1.1–5.1, $p = 0.04$). These associations remained after adjusting for age and BMI (insulin deficiency: OR 6.5, 95% CI 2.6–16.4, $p < 0.01$; GAD antibodies: OR 2.4, 95% CI 1.0–5.4, $p = 0.04$). Other baseline antibodies (IA2, IAA, Zn-T8) had no association. GAD antibodies were more common in insulin deficient (54.6%) than non-insulin deficient (4.9%) participants.

Conclusion: In patients with type 2 diabetes, insulin deficiency, possibly due to islet cell autoimmunity, is associated with death and a history of severe hypoglycemia. Whether treatments to preserve insulin secretion in type 2 diabetes may reduce mortality remains an intriguing area for further study.

Clinical Trial Registration Number: NCT00000620

Supported by: NIDDK and NHLBI

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Is hypoglycaemia an increasing barrier to achieving HbA1c target for NPH insulin than insulin glargine?Q. Zhang¹, E. Chou¹, H.-W. Chung², H. Wang¹;¹R&D, Sanofi, Bridgewater, ²TechData, King of Prussia, USA.

Background and aims: Hypoglycemia is a common side effect of insulin therapy and may impede the outcomes of glycemic control. This study was to assess risk of hypoglycemia at each level of achieved A1C between patients initiating NPH insulin (NPH) and insulin glargine (GLA) as add-on to oral anti-diabetic drugs (OAD) from a US managed care database.

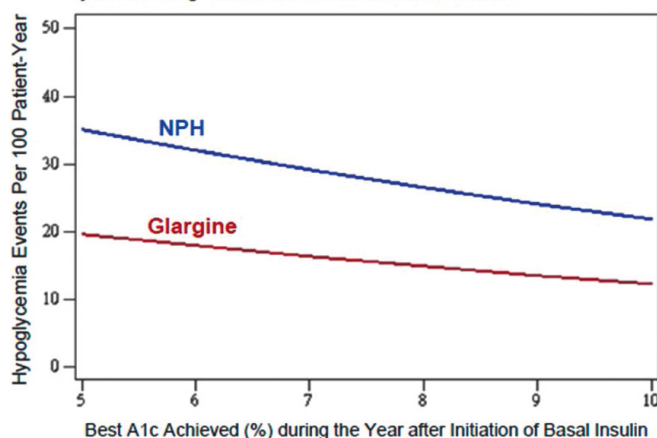
Materials and methods: Patients with T2DM who initiated NPH or GLA between January 2006 and December 2012 were identified from Clinformatics™ DataMart Multiplan. All patients had a minimum of 1-year continuous health coverage prior to and after basal insulin initialization. Baseline comorbidity was measured by Charlson Comorbidity Index (CCI) with a higher number corresponding to increased burden of comorbidity. Number of hypoglycemic events was evaluated against achieved level of A1C and tested at each A1C level with adjustment of baseline patient characteristics.

Results: NPH ($n = 864$) had mean age of 47 years with average baseline A1C of 7.5% and CCI of 2.38 relative to GLA ($n = 10,843$) with mean age of 55 years, baseline A1C of 8.6% and CCI of 2.55. Both groups had about 46% female.

During the year following basal insulin initiation, unadjusted achieved A1C decreased -1.0% from baseline in NPH and -1.2% in GLA. Mean number of hypoglycemic events in the year was 0.74 in NPH and 0.50 in GLA after adjustment of baseline characteristics. Overall, number of hypoglycemic events increased as achieved A1C during the year approached the target ($\leq 7\%$) and remained higher in NPH relative to GLA ($p < 0.01$). In particular when achieved A1C was at 7%, number of hypoglycemia events was estimated at 29.1/100 patient-years in NPH and 16.3/100 patient-years in GLA (Fig 1).

Conclusion: Consistent with previous research, this study shows an elevated hypoglycemia risk in NPH relative to GLA in the US managed care database. Furthermore, the risk of hypoglycemia rises as A1C approaches 7%, suggesting hypoglycemia as an increasing barrier for more intensive glycemic control in patients with T2DM initiating basal insulin from the clinical practice setting.

Fig 1. Number of hypoglycemic events over achieved A1C in the year following the initiation of basal insulin initiation



Supported by: Sanofi

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Is the fear of hypoglycaemia associated with glycaemic imbalance?

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Background and aims: The fear of hypoglycemia in clinical practice is important, but few practical tools exist to evaluate it. The HFS-II (Hypoglycemia Fear Survey-II) is a validated questionnaire measuring fear of the risk of hypoglycemia in patients with diabetes. It evaluates behaviors to avoid hypoglycemia and its consequences as well as degree of worry related to hypoglycemia. The objective of this analysis is to estimate the clinical correlations of a high HFS score ($> +1SD$) on high HbA1c value ($\geq 7\%$) and occurrence of severe hypoglycemia within 30 days.

Materials and methods: Data from 1927 French patients with insulin-treated diabetes (1008 patients with type 1 diabetes mellitus [T1DM] and 919 with type 2 diabetes mellitus [T2DM]) completing the HFS-II questionnaire in the DIALOG observational prospective study was used. The correlated factors were analyzed using a multivariate logistic regression model.

Results: Factors that were correlated to a HbA1c $\geq 7\%$ were separately investigated in patients with T1DM and T2DM. For patients with T1DM, the main factors contributing to an HbA1c $\geq 7\%$ were the HFS class $> +1SD$ (odds ratio [OR] 3.5; 95% confidence interval [95CI] 1.9–6.4) and the class between -1 and +1SD (OR 2.8; 95CI 1.7–4.7) versus the lowest class ($< -1SD$), as well as the insulin therapy duration > 10 years (OR 1.8; 95CI 1.3–2.5). In patients with T2DM, the HFS score was no longer significant in the multivariate model. On the other hand, the main associated factors contributing to occurrence of a severe hypoglycemic event in both T1DM and T2DM patients were history of hypoglycemia (OR 3.8; 95CI 1.4–10.6), T1DM (OR 1.8; 95CI 1.3–2.5) and an HFS score $> +1SD$ versus $< -1SD$ (OR 7.1; 95CI 3.2–16.1) or between -1SD and +1SD versus $< -1SD$ (OR 3.4; 95CI 1.6–7.5).

Conclusion: The fear of hypoglycemia is correlated with high HbA1c values in patients with T1DM and also with the occurrence of severe hypoglycemia in both T1DM and T2DM.

PS 043 Nocturnal and drug-induced hypoglycaemia

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Predisposing factors for hypoglycaemia: analysis from the SAVOR-TIMI 53 trial

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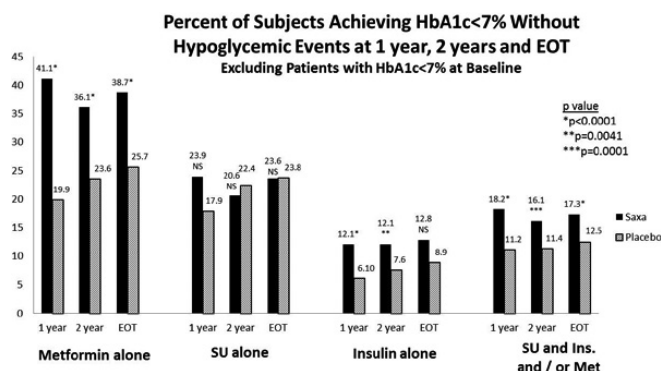
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Background and aims: Hypoglycemia is a significant barrier to treatment escalation by patients (pts) and physicians. Predisposing factors to hypoglycemia in the SAVOR-TIMI 53 trial were studied.

Materials and methods: In the SAVOR-TIMI 53 trial, 16,492 patients (pts) with type 2 diabetes (T2D) were randomized to receive either saxagliptin (SAXA) or placebo. Conventional therapy was adjusted by investigators to achieve HbA1c (A1c) target. Pts were followed for a median of 2.1 years. Patients were requested to record hypoglycemic episodes based on symptoms suggestive of hypoglycemia that recovered by carbohydrate ingestion and/or documentation of low blood glucose < 54 mg/dl (minor) or the need for assistance from another person (major).

Results: 2,722 individuals (16.5% of trial participants) experienced at least one hypoglycemic episode and 312 (1.9%) experienced at least one major hypoglycemic episode. Hypoglycemic risk increased with age, disease duration, reduced GFR and increased albuminuria ($p < 0.01$). Incidence of hypoglycemic events changed with baseline A1c: 12.8% at A1c $< 7\%$, 17.4% at A1c 7–8%, 20.5% at A1c 8–9% and 16.3% at A1c $> 9\%$. Pts on sulfonylurea (SU), long acting insulin (glargine or detemir), intermediate acting insulin, or short acting insulin in different combinations experienced increased hypoglycemic rates (14.9%, 20.2%, 23.9% and 29.3% respectively). Pts assigned to SAXA vs. placebo reported more hypoglycemic events (17.6% vs. 15.4%, $p < 0.001$), including major hypoglycemic events (2.1% vs. 1.7%, $p = 0.05$). Hypoglycemia requiring hospitalization was similar between groups (0.6% vs. 0.5%, $p = 0.3$). Higher rates of hypoglycemia in SAXA vs. placebo arm were observed in SU treated pts (alone or in combination with other oral anti diabetic drugs) (17.2% vs. 12.5%, $p < 0.001$), but not in insulin treated pts (27.1% vs. 26.7%, $p = 0.8$). Patients treated with SU and baseline A1c $< 7\%$ in SAXA arm had increased rates of major hypoglycemia (3.6% vs. 1.6%, $p = 0.02$). Addition of SAXA vs. placebo to metformin alone, insulin alone or to SU in combination with insulin and/or metformin, resulted in more pts achieving A1c $< 7\%$ at 1 year without experiencing hypoglycemia (figure).

Conclusion: The SAVOR-TIMI 53 trial enables us to characterize the main predictors for development of hypoglycemia in T2D pts at high cardiovascular risk. Adding SAXA to metformin alone, insulin alone or to SU in combination, increases the likelihood of attaining a glycemic target of A1c $< 7\%$ without excess hypoglycemic risk.



Clinical Trial Registration Number: NCT01107886

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A simple algorithm to predict the risk of hypoglycaemia in case of physical activity (PA) in patients with type 1 diabetes under pump therapy

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Background and aims: The aim of our study was to develop a model to predict the occurrence of hypoglycemic events from data of Continuous Monitoring Systems (CGMs), collected during physical activity (PA) sessions, in patients with type 1 diabetes (T1D), under pump therapy.

Materials and methods: Twenty adult patients with type 1 diabetes (11 males, mean age 45.3±11.9 yrs, HbA1c 7.9±0.9%, VO2max 33±10 ml/kg/min) performed 5 basal sessions (i.e. 3 hours after lunch) in a random order: 4 PA sessions (30-min cycling sessions on a constant-power electronic cycle ergometer) at 2 levels of PA and with 2 kinds of adjustment of the basal rate (BR) of their pump (50% VO2max with a BR reduction of 50 or 80%, 75% VO2max with again a BR reduction of 80 or 100%) and 1 rest session. Patients wore a CGMs from the beginning of each session and until the next morning. Classification methods were used, based on the patients' profile and the kind of sessions, to classify patients according to the occurrence or not of hypoglycemia. Several models of prediction were then elaborated. A number of explanatory variables had been previously selected and were used to compare the effectiveness of each model to predict the risk of hypoglycemia.

Results: From one model to another, variables that proved not to be essential or that were not available for the patient in a realistic context, were progressively removed. Finally, we considered variables available during the first ten minutes of a PA: weight, age, gender, VO2max, BR reduction, glucose level at the beginning of PA, glycemic gradient during the first 10 min of PA. Among these variables, we used statistical model selection methods in order to select the most relevant variables for the prediction of hypoglycemic events which were weight, and glycemic gradient during the first 10 min of PA reported to the initial glucose level (GG10). The value of the Area Under the Curve (AUC) of the ROC curve based on these variables was 0.74 which means a rather acceptable prediction but that can still be improved. We used a logistic regression for modeling the probability *p* of occurrence of hypoglycemic events and the estimated model is: $\text{logit}(p) = -4.13 - 438.7 \times \text{GG10} + 0.5 \times \text{weight (kg)}$, where $\text{logit}(p) = \log(p/(1-p))$ is an increasing function of *p*. The model, based on these 3 variables easy to collect in clinical practice, provides a prediction rate exceeding 80% (concordance between the predicted and the actual situation) with less than 20% of mismatch data.

Conclusion: A simple model of prediction mainly based on the CGM gradient of the first 10 min of a 30-min physical activity session, allows to predict the occurrence of hypoglycemia with a relative good efficiency. This kind of model that we can imagine coupled with CGMs to predict the risk of hypoglycemic event in clinical practice, (open-loop system), may, in an improved version, reveal to be of particular interest in the development of closed-loop systems.

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Supported by: EFSD, Medtronic

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The early warning indicators for nocturnal hypoglycaemia in type 2 diabetes

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Background and aims: The existing research on hypoglycaemia is largely done in special environments such as use of a specific medicine or focusing on a certain age period, its practicality in broad clinical contexts is thus limited. Moreover, most causes of hypoglycaemia like endogenous insulin inadequacy and the diabetic process cannot be altered. This study is to explore quantitative indicators warning of nocturnal hypoglycaemia by using Continuing Glucose Monitoring System (CGMS) on a large sample in routine clinical environments to seek some basis for preventing hypoglycaemia in clinics.

Materials and methods: First, 1147 patients (male 723 and female 424) of type 2 diabetes were sampled, whose ages being 59.21±11.31(14~91 years old) and Mean Blood Glucose (MBG) being 7.41±2.98 mmol/L. Those

whose BGs turned stable after treatment, except those who took medicine like hypnotics or sedative before sleep, were monitored for 65 to 82 hours by CGMS. Second, another 479 patients whose BG 3 hours after supper was ≤ 4.7mmol/L were sampled and randomly divided into the intervention group and the non-intervention group, measures like insulin dosage reduction before sleep and/or eating some 50 to 100 grams of protein or carbohydrate were adopted for the intervention group. Statistical analyses were made by correlation regression (with *r* below representing correlation coefficient), relative risk (RR) calculation and Chi-square test whereas comparison between the two groups was made by *t* test.

Results: The occurrence rate of hypoglycaemia, asymptomatic hypoglycaemia and nocturnal hypoglycaemia is respectively 37.23% or 427 cases, 22.75% or 261 cases, and 18.31% or 210 cases. Among 965 person-times showing hypoglycaemia, 60.8% or 697 person-times were asymptomatic whereas 49.2% or 470 person-times showed nocturnal hypoglycaemia. 1. MBGs are correlated negatively with incidence rates of hypoglycemia on an exponential curve ($r = -0.995$, $Y_e = 10^{2.550 - 0.139X}$) and negatively with incidence rates of nocturnal hypoglycaemia ($r = -0.953$, $Y_e = 10^{4.135 - 3.445 \lg X}$) and of asymptomatic hypoglycaemia ($r = -0.963$, $P < 0.01$, $Y_e = 10^{3.663 - 2.718 \lg X}$) on a hyperbolic curve. 2. The risk occurrence of nocturnal hypoglycaemia during 22:00~2:00 is 1.72 times that during 2:00~6:00 ($RR = 1.72$, $\chi^2 = 31.667$, $P < 0.01$). 3. BGs 3 hours after supper are negatively correlated with incidence rates of nocturnal hypoglycaemia on an exponential curve ($r = -0.955$, $Y_e = 10^{3.450 - 2.627 \lg X}$), with the limit value of BGs 3 hours after supper for 50% of nocturnal hypoglycaemia incidence rates being 4.7mmol/L. 4. For the 479 patients, no significant difference is found between MBGs of the two groups (8.29 ± 1.98 vs 8.53 ± 2.24 , $t = 1.182$, $P > 0.05$). The incidence rate of the intervention group is significantly reduced in contrast to that of the non-intervention group (9.67% vs 16.76%, $\chi^2 = 8.79$, $P < 0.01$), with $RR = 1.7$, relative efficiency = 42.43%, $\chi^2 = 6.16$, $P < 0.05$.

Conclusion: 1. Occurrence rates of nocturnal hypoglycaemia in routine clinical environments are high. 2. 22:00~2:00 is the peak period when nocturnal hypoglycaemia occurs. 3. The BG 3 hours after supper being ≤ 4.7mmol/L can be used as a quantitative indicator warning of the occurrence of nocturnal hypoglycaemia. 4. The occurrence of hypoglycaemia in the non-intervention group was 1.7 times that in the intervention group; with interventions to patients whose BG 3 hours after supper were ≤ 4.7mmol/L, 40% of them were protected from nocturnal hypoglycaemia.

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The impact of nocturnal hypoglycaemia on sleep in subjects with type 2 diabetes

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Background and aims: Nocturnal hypoglycaemia is a major barrier in achieving optimal glycaemic control in patients with diabetes. Knowledge about the consequences of nocturnal hypoglycaemia on sleep is limited. The aim of the present trial was to investigate the impact of nocturnal hypoglycaemia on sleep pattern.

Materials and methods: In this randomised, single-blinded, two-period, cross-over trial, 26 subjects with type 2 diabetes attended two experimental night visits (one normoglycaemic and one hypoglycaemic) in randomised order to assess the impact of nocturnal hypoglycaemia on sleep (using polysomnography) and on hormonal responses. Plasma glucose (PG) levels were controlled on the experimental nights by hyperinsulinaemic glucose clamping. On the hypoglycaemic night, hypoglycaemia was induced when subjects had reached sleep stage N2 or deeper by turning off the glucose infusion (PG target: 2.7–2.8 mmol/L) for approximately 15 min, after which subjects were brought back to normoglycaemia. On the normoglycaemic night, PG was maintained at 5–7 mmol/L throughout the night.

Results: There was no difference between the hypoglycaemic night and the normoglycaemic night, in either the number of EEG-identified arousals or awakenings in the first 4 hours of sleep (0–4h after reaching sleep stage N2). During the last 4 hours (4–8h) and during the entire night (0–8h), the number of awakenings was significantly lower ($P < 0.05$) on the hypoglycaemic night than on the normoglycaemic night (observed geometric means 4–8 h: 10 vs. 14 awakenings and 0–8h: 25 vs. 30 awakenings). Total sleep time tended to be longer on the hypoglycaemic night (observed means: 366 vs. 349 min, $p = \text{NS}$).

Statistically significantly higher hormonal counter-regulatory responses (adrenaline, growth hormone and cortisol) to hypoglycaemia were observed as compared with normoglycaemic night.

Conclusion: Nocturnal hypoglycaemia in patients with type 2 diabetes caused a decrease in awakening response following the event. These findings underscore the risks associated with nocturnal hypoglycaemia since these events potentially affect the subject's ability to wake up and respond adequately to hypoglycaemia.

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Impact of nocturnal hypoglycaemia on the risk of hypoglycaemia the next day

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Background and aims: In people with type 1 diabetes [T1D], nocturnal hypoglycaemia (NH) is reported to occur on about 30% of nights. Fear of [NH] can lead to suboptimal glucose control. Antecedent hypoglycaemia, including specifically at night, blunts symptomatic and hormonal responses to subsequent hypoglycaemia, which may lead to a vicious cycle of recurrent hypoglycaemia, ultimately in some patients leading to impaired awareness of hypoglycaemia [IAH]. We aimed to study the impact of biochemical NH on the risk of subsequent daytime hypoglycaemia [DH], and conversely also the impact of antecedent DH on the risk of subsequent NH in adults with T1D using continuous glucose monitoring [CGM].

Materials and methods: 172 consecutive CGM traces obtained using Medtronic iPRO2 met the following criteria: 48 hours of CGM data starting from midnight, ≥ 3 valid calibrations/24 hours, mean absolute error <18 . Patient inclusion criteria included: clinical diagnosis of T1D and not pregnant. We defined moderate CGM hypoglycaemia as sensor glucose <3.9 mM and severe CGM hypo as sensor glucose ≤ 2.2 mM for at least 20 minutes. Nocturnal was defined as 24:00–06:00 hrs.

Results: The cohort providing data was 40% male, mean age 44.75 (± 14.54) yrs; duration of T1D 18.95 (± 13.821) yrs, mean HbA1c 8.1% (± 1.30) [65 ± 10.2 mmol/mol]. 29.8% used continuous subcutaneous insulin infusion [CSII]; 41.0% had IAH. 20.9% of traces had at least one episode of NH <3.9 mM and 4.7% had at least one episode of NH ≤ 2.2 mM in the 48 hrs. Risk of mild DH <3.9 mM following NH <3.9 mM was 48.7% compared to 43% after no NH <3.9 mM [χ^2 0.537] giving a relative risk [RR] of 1.13 [95%CI 0.77–1.66; $p=0.124$]. However, 25% of those with severe NH ≤ 2.2 mM had a subsequent DH ≤ 2.2 mM, compared to 6.7% risk of severe DH ≤ 2.2 mM after no NH ≤ 2.2 mM [χ^2 0.056] giving a RR of 3.72 [CI 0.99–14.08; $p=0.052$]. Conversely, risk of mild NH <3.9 mM after DH <3.9 mM was 30.3% vs. 12.5% if no DH <3.9 mM [χ^2 0.004] giving a relative risk of 2.42 [95%CI 1.29–4.54; $p=0.006$]. Risk of severe NH ≤ 2.2 mM after DH ≤ 2.2 mM was 6.6% vs. 1.8% if no DH [χ^2 0.001] giving a RR of 7.34 [CI 1.97–27.33; $p=0.003$]. Frequency of mild NH <3.9 mM was significantly lower in CSII vs. MDI (10% vs. 25%, $p=0.047$), with less time hypo [14 vs. 52 mins/night]; $p=0.035$ mins]. There were no significant differences between number or duration of mild DH episodes between MDI and CSII, or in number or duration of severe DH or NH ≤ 2.2 mM. Frequency of NH was not significantly different between normal awareness and IAH [NH <3.9 mM 0.29 vs. 0.25 $p=0.620$; NH ≤ 2.2 mM [0.05 vs. 0.06 $p=0.824$]. Duration of NH was also not significantly different; NH <3.9 mM [52 vs. 40 mins; $p=0.504$], NH ≤ 2.2 mM [5 vs. 5 mins; $p=0.936$].

Conclusion: Mild NH does not increase the risk of mild hypoglycaemia the following day, with a trend to increased risk of DH after more severe NH. However, both mild and severe DH significantly increases the risk of NH. The lack of difference in hypoglycaemia rate using CGM between those with impaired awareness and normal awareness needs further research. Our data also add to the body of evidence that CSII use is associated with significantly reduced frequency and duration of NH. Meanwhile, patients can be advised to reduce overnight insulin following episodes of daytime hypoglycaemia.

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Severe hypoglycemia during therapy with sulfonylurea in patients with type 2 diabetes (T2D) in Germany/Austria: event rate and identification of patients at risk

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Background and aims: We investigated the rate of severe hypoglycemia and confounding factors in T2D patients treated with sulfonylurea (SU) in the German/Austrian DPV-Wiss-database cohort.

Materials and methods: Data from 29,485 SU-treated patients were analyzed (mean (SD) age 69.5(11.5) years, diabetes duration 9.7 (7.6) years, with/without antidiabetic co-medication). Primary objective was to estimate the event rate of severe hypoglycemia (defined as requirement for help +/- coma, or emergency hospitalization due to hypoglycemia). Secondary objective was the identification of confounding risk factors for hypoglycemia through group comparison and hierarchical negative binomial regression.

Results: Severe hypoglycemic events were reported in 826 (2.8%) of all SU-treated patients; $n=531$ (1.8%) suffered from coma, $n=501$ (1.7%) were hospitalized at least once. Event rates/year [95%CI] were 0.039 [0.037;0.042], 0.019 [0.018;0.021] and 0.016 [0.015;0.018]. Unadjusted severe hypoglycemia rates for different treatment groups were 0.074 (SU + insulin), 0.038 (SU + insulin + other oral antidiabetic agents [OaD]), 0.027 (SU + other OAD), 0.042 (SU only). Patients with severe hypoglycemia were older ($p<0.0001$) and had longer diabetes duration ($p=0.0195$). In regression analysis, severe hypoglycemia was associated with lower HbA1c and with the frequency of mild hypoglycemic events (all $p<0.0001$). Lower eGFR was associated with an increased rate of severe hypoglycemia (>60 ml/min: 3.9% of all patients with SU; 30–60 ml/min: 4.8%; ≤ 30 ml/min: 7.7%). Indirect measures of insulin resistance such as increased BMI, plasma triglycerides and participation in educational diabetes program were associated with less frequent severe hypoglycemia (all $p\leq 0.0002$).

Conclusion: Our analysis of real-life data showed a higher risk for severe hypoglycemia in older patients, in patients reporting more non-severe hypoglycemia and in patients with decreased eGFR, suggesting that treatment with SU in those patients should be considered with caution.

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High prevalence of silent hypoglycaemic episodes among patients with well-controlled type 2 diabetes mellitus treated by sulfonylureas

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Background and aims: Hypoglycaemia is a well known cardiovascular risk factor. The aim of this study was to determine the prevalence of silent hypoglycaemic episodes among patients with well-controlled type 2 diabetes treated with sulfonylurea.

Materials and methods: 10 type 2 diabetic patients (7 men, 3 women) with HbA1c $\leq 7\%$ treated with sulfonylurea and/or metformin without symptoms of hypoglycaemia during the past two years were included in the study (mean age: 71 ± 10 , mean duration of diabetes mellitus 11 ± 9 years, mean HbA1c: $4.9 \pm 0.9\%$). A 120-h long (5 days) glucose monitoring was performed using a Continuous Glucose Monitor System (CGMS) device (Medtronic iPro2[®]). Moreover, patients were asked to record four self-monitored capillary blood glucose levels each day for calibration of the monitor and also to record meal times, exercise and symptoms of hypoglycaemia. According to EASD/ADA recommendation a glucose concentration of ≤ 3.9 mmol/l can be used as a cut-off value in the classification of hypoglycaemia. The number of hypoglycaemic events ≤ 3.9 mmol/l and, additionally, the number of hypoglycaemic

events <3.1 mmol/l were determined. An event was defined as a glucose value that persisted for at least 15 minutes with or without symptoms.

Results: Ten patients were each monitored for an average of 5757.0 ± 1192 min. The mean interstitial glucose (IG) concentration was 6.10 ± 0.56 mmol/l. The minimum IG was 2.2 mmol/l and maximal IG concentration was 13.3 mmol/l. 7/10 patients had a total number of 30 silent hypoglycaemic episodes (interstitial glucose ≤ 3.9 mmol/l), mean: 4.6 ± 2.6 episodes per patients. The total duration of these hypoglycemic episodes was 374.5 ± 462.97 min. 5/10 patients had hypoglycaemic episodes of interstitial glucose ≤ 3.1 mmol/l. The numbers of episodes were 2.0 ± 0.7 per patient. The total number of episodes was 9. The total duration of hypoglycemic episodes with glucose values ≤ 3.1 mmol/l was 96.0 ± 156.55 min. No symptoms of hypoglycemia were recorded by any patient in their daily diaries. CGMS measurements were generally well tolerated.

Conclusion: Our data suggest that silent hypoglycemia is quite common in well-controlled patients with type 2 diabetes treated with a sulfonylurea with or without metformin. It should be noted that glycemic control of our patients was overly normal. Nonetheless, as a key finding, in our small cohort of patients all episodes of hypoglycaemia were silent. CGMS might serve as a useful clinical tool to discover silent hypoglycemia.

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In case of physical activity performed just after lunch, what is the best option to reduce the risk of hypoglycaemia in case of pump therapy: basal rate or bolus reduction?

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Background and aims: In T1 diabetic subjects on pump therapy, there is no consensus regarding the adjustment of insulin doses in the event of physical activity (PA) just after a meal. The aim of our study was to determine what was the best attitude in such a situation between reducing the basal rate (BR) of the pump (BRR) or the prandial bolus (PBR) to limit the risk of hypoglycemia.

Materials and methods: 20 patients (11 men, BMI 24 ± 5 kg/m², age 45 ± 12 yrs, HbA1c $7.9 \pm 0.7\%$, diabetes duration 17.6 ± 10 yrs, on pump therapy for 5 yrs, practicing 4.3 hrs/wk of PA) performed 2 physical activity sessions (30-min cycling sessions) 90 min after lunch in a random order, at 50%VO₂max, one with bolus reduction (PBR) of 30/50%, the other one with basal rate (BRR) reduction of 50/80% (during PA+2hrs). Blood glucose (BG) levels were measured during the exercise period and over the following 2hrs. Patients wore an iPro2 device from lunchtime until breakfast the next day. We focused on the occurrence of hypoglycemic events recorded on the CGM system.

Results: CGM level at lunchtime did not differ between both groups but was higher after lunch, in case of PBR vs BRR, as was glucose level just before exercising [221 ± 62 vs 171 ± 59 mg/dl]. The mean decrease in glucose value was similar in PBR vs BRR (BG = -71 vs -77 mg/dl, CGM = 42.1 vs 34.4). After the initial drop, the average glucose level remained stable during the afternoon, at a higher level in case of PBR vs BRR. Total glucose AUC and time spent in $[70;180$ mg/dl] did not significantly differ in both cases but less time was spent >180 mg/dl ($p=0.057$) in case of PBR compared to BRR. Only 1 hypoglycemia occurred during PA (BRR). During the afternoon, there was a trend towards less hypoglycemic events in case of PBR vs BRR (3 events in 3 patients vs 10 in 6, $p=0.07$) with a mean number of events 3.5 times lower (0.16 ± 0.37 vs 0.56 ± 0.92). There was no significant hyperglycemic rebound before dinner in both cases and no significant difference in the CGM level at bedtime (176 ± 79 mg/dl vs 160 ± 85 , $p=NS$). The average glucose level remained stable and at a similar level at night in both cases. There was no difference in the timing and in the level of nocturnal nadir between PBR and BRR [77 ± 35 vs 77 ± 34 mg/dl, $p=NS$]; no differences were observed in the total AUC, the time spent in or out the range $[70;180]$ or $[80;140]$. At night, the number of hypoglycemic events did not differ in case of PBR vs BRR (7 events in 5 patients vs 10 events in 7 patients, $p=0.39$) and the mean number did not significantly differ (0.37 ± 0.68 vs 0.56 ± 0.86). There was no hyperglycemic rebound the next morning, even in case of hypoglycemia at night.

Conclusion: In case of PA 90 min after a meal, PBR led to a higher sustainable glucose level in the afternoon than in case of BRR, limiting therefore the risk of hypoglycemia. PBR can thus be considered as the security option while BRR would lead to a lower glucose level and potentially to an increased

risk of hypoglycemia. However, in both cases the practice of PA will first lead to an initial drop in glucose level of around 70 mg/dL. If a BRR strategy is chosen, then the BRR should be anticipated at least 30 min earlier to limit the risk of hypoglycemia.

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Effects of automatic insulin pump interruption and bedtime glucose levels on nocturnal hypoglycaemic events in the ASPIRE In-Home Study
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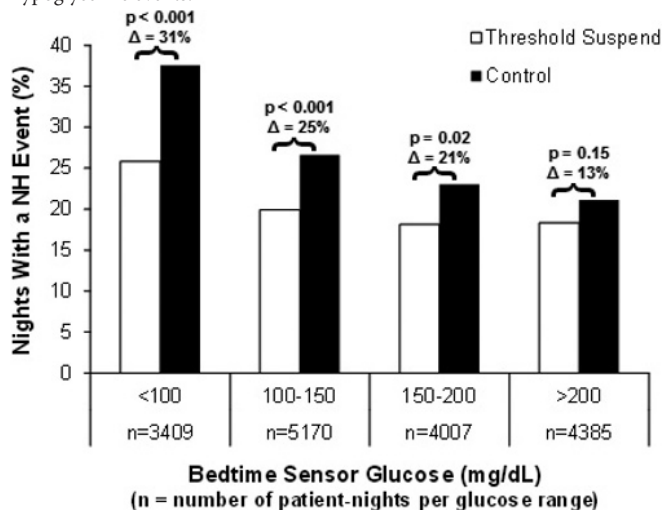
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Background and aims: The ASPIRE In-Home Study was designed to evaluate the Threshold Suspend (TS) feature of sensor-augmented pump (SAP) therapy, which automatically interrupts insulin delivery at a pre-set sensor glucose (SG) threshold. We evaluated the risk of nocturnal hypoglycemia (NH) with regard to bedtime sensor glucose (SG) values and use of the TS feature.

Materials and methods: Nocturnal hypoglycemia (NH) was defined as an event lasting >20 min with SG values ≤ 65 mg/dL that started between 10:00 PM and 8:00 AM. Subjects with type 1 diabetes with ≥ 2 NH episodes during a 2-week run-in phase were eligible. A total of 247 patients were randomized to SAP therapy with the TS feature (TS group, $n=121$) or without the TS feature (Control group, $n=126$). During the 6-month study phase, each patient-night was classified according to the patient's treatment group assignment and the 10:00 PM ("bedtime") SG value. Four categories of bedtime SG values were defined as <100 mg/dL, 100–150 mg/dL, 150–200 mg/dL, and >200 mg/dL. The percentage of nights with a NH event was calculated for the TS and Control groups within each bedtime SG value category.

Results: The figure shows the percentage of nights with a NH event according to treatment group and bedtime SG value, and the number of patient-nights in each SG category. Higher bedtime SG values tended to decrease the risk of NH in the Control group ($p<0.001$ for trend) and in the TS group ($p=0.01$ for trend). Subjects in the TS group who had bedtime SG values <100 mg/dL, 100–150 mg/dL, or 150–200 mg/dL were significantly less likely to experience a hypoglycemic event later that night than subjects in the Control group with similar bedtime SG values ($p<0.001$ for bedtime SG values <150 mg/dL; $p=0.02$ for bedtime SG values 150–200 mg/dL). For bedtime SG values <100 mg/dL, the TS group's percentage of nights with a hypoglycemic event was 31% less than in the Control group, and 25% less for bedtime SG values of 100–150 mg/dL. When the bedtime SG value was >200 mg/dL, the difference between the groups was not significant.

Conclusion: In subjects with type 1 diabetes and bedtime SG values <200 mg/dL, use of the TS feature was associated with a lower risk of nocturnal hypoglycemic events.



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PS 044 Endocrine control of glucose tolerance

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Association of low normal TSH levels and increment of TSH levels with the development of metabolic syndrome; a 6-year retrospective, longitudinal study

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Background and aims: Many cross-sectional studies have showed an increased association with metabolic syndrome (MetS) in the subjects with higher TSH levels in subjects with euthyroid (0.3-6.5mU/l) and subclinical hypothyroid (6.5-10mU/l). However, little is known about the relationships between TSH levels and development of MetS in a longitudinal study. We investigated the associations of baseline TSH levels or increment of TSH levels with new-onset MetS in subjects with euthyroid and subclinical hypothyroid.

Materials and methods: We retrospectively analyzed the medical records of 23717 subjects (13989 men and 9728 women, age 18 to 89 years) who underwent health screening annually or biennially from 2006 to 2012 at our Medical Center (Seoul, Republic of Korea). After exclusion of the subjects with abnormal value of T4 or fT4 and subjects with MetS at baseline, we selected 19823 subjects (13989 men and 9728 women). We divided the subjects into 3 groups by baseline TSH levels as follows; low-normal TSH group (0.3-2.5mU/l), high-normal TSH group (2.5-6.5mU/l) and subclinical hypothyroid TSH group (6.5-10mU/l). We also divided the subjects into two groups by changing TSH levels from baseline to last visit (increasing TSH group; the ratio of last TSH/initial TSH ≥ 1 , decreasing TSH group; the ratio of last TSH/initial TSH < 1).

Results: By using a Cox proportional hazard models, the groups by baseline TSH level and the groups by a change of TSH levels, were analyzed for time to the development of MetS after adjustment of age, smoking, body mass index, systolic and diastolic blood pressure, lipid profile, fasting blood glucose and hemoglobin A1c. Compared to high-normal TSH group and subclinical hypothyroid group, the prevalence of new-onset MetS was higher among subjects with low-normal TSH group after adjustment ($p < 0.001$) in men, but not women. Compared to decreasing TSH group, increasing TSH group had an increased hazard ratio of 1.26 (95% confidence interval, 1.19-1.35, $p < 0.001$) in men and 1.23 (95% confidence interval, 1.09-1.37, $p < 0.001$) in women.

Conclusion: In contrast to the cross section study, the low-normal TSH levels (0.3-2.5mU/l) may be a risk for development of MetS in men, and increment of TSH levels also could be an independent risk for new-onset MetS in men and women.

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Glucose intolerance and diabetes in patients with new onset Graves' hyperthyroidism

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Background and aims: Overt thyrotoxicosis is associated with reduced insulin sensitivity (IS) and beta cell function, the prevalence and characteristics of diabetes and impaired glucose regulation (IGR) in patients with Graves' hyperthyroidism is still unknown in Chinese. This study is to evaluate the glucose homeostasis in patients with Graves' hyperthyroidism and the associations with thyrotropin levels.

Materials and methods: From 2012 to 2014, a total of 278 patients with new onset Graves' hyperthyroidism without anti-thyroid therapy were enrolled from the department of endocrinology of our university. The oral glucose tolerance test (OGTT) was performed to measure the levels of fasting and 2-hour postprandial blood glucose (FBG and 2hPG) levels. In addition, the glycosylated hemoglobin (HbA1c) and thyroid hormones levels were also measured. Analyses were performed with SPSS software version 13.0.

Results: Among the 278 patients with Graves' hyperthyroidism, 82 cases is male (29.5%), and 196 cases were female (70.5%), with an average age of 45.4 years. There were 148 cases (53.2%) with normal glucose tolerance (NGT), 88 cases (31.7%) with IGR and 42 cases (15.1%) with diabetes. The T3 ($P < 0.05$) and FT3 ($P < 0.01$) levels were significantly higher in patients with diabetes than with NGT, after adjusting for sex, age and BMI. By the tertile groups

of fT3, the levels of FBG ($P<0.05$), 2hPG ($P<0.01$), HbA1c ($P<0.05$) in the 3rd tertile group were significantly higher than the 1st tertile group, after sex, age and BMI were adjusted. The levels of FBG ($r=0.24$, $P=0.05$) were positively correlated with serum fT3 levels, and 2hPG were positively correlated with serum T3, T4, fT3 levels ($r=0.30$, $P=0.01$; $r=0.28$, $P=0.02$; $r=0.30$, $P=0.02$, respectively). Multivariate linear regression model showed that only BMI was independently associated with FBG, but BMI and fT3 were both independently associated with 2hPG in these patients. Logistic regression model showed that BMI (OR=1.26, 95% CI 1.00–1.60, $P=0.05$), fT3 (OR=1.53, 95% CI 1.06–2.21, $P=0.03$) were the independent risk factor of diabetes in patients with Graves' hyperthyroidism.

Conclusion: The patients with Graves' hyperthyroidism had a high percentage of abnormal glucose homeostasis, including IGR and diabetes. Thyroid hormone was closely related to glucose homeostasis, BMI and fT3 were the independent risk factor for 2hPG and diabetes in the patients with Graves' hyperthyroidism.

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Association between lower normal free thyroxine concentrations and obesity phenotype in healthy euthyroid subjects

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Background and aims: We investigated whether thyroid function could identify obesity phenotype in euthyroid subjects.

Materials and methods: A cross-sectional analysis was performed among non-diabetic, euthyroid subjects. We stratified subjects into four groups by BMI and insulin resistance (IR), defined by a HOMA-IR in the top quartile.

Results: Of 6241 subjects, 33.8% were overweight or obese (OW/OB) and 66.2% were normal weight (NW). Free thyroxine (FT4) levels were negatively associated with body mass index, waist circumference, triglyceride, c-reactive protein and HOMA-IR and positively with high-density lipoprotein cholesterol in both genders. In multivariate regression analysis, FT4 level, a continuous measurement, was negatively correlated with HOMA-IR ($\beta = -0.155$, $P < 0.001$ in men; $\beta = -0.175$, $P < 0.001$ in women). After adjustment for age, sex, metabolic and life style factors, subjects in the lowest FT4 quartile had an odds ratio (OR) for IR of 1.99 (95% confidence interval 1.61–2.46), as compared to those in the highest quartile. The association between low FT4 and IR remained significant in both NW and OW/OB subgroups.

Conclusion: Low normal FT4 levels were independently related to IR in NW and OW/OB euthyroid subjects. Further studies are needed to investigate the mechanisms by which low FT4 levels are linked to high IR in euthyroid ranges.

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Exogenous glucagon decreases IGF-I bioactivity in humans, independently of insulin levels, by modulating its binding proteins

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Background and aims: The mechanisms underlying glucagon-induced growth hormone (GH) release are unclear. Our goal was to identify the role of glucagon-induced changes in IGF-I bioactivity as a possible mechanism, excluding changes in insulin as known major confounder.

Materials and methods: In our double-blind placebo-controlled study, we investigated changes in GH, IGF binding proteins (IGFBPs) and IGF-I bioactivity, determined using the cell-based KIRA-method, after intramuscular glucagon administration in 13 patients with type-1 diabetes mellitus (T1DM; 6 males; [BMI]: 24.8 ± 0.95 kg/m²), 11 obese participants (OP; 5/6; 34.4 ± 1.7

kg/m²), and 13 healthy lean participants (LP; 6/7; 21.7 ± 0.6 kg/m²). In vitro, the impact of glucagon on forkhead box transcription factor O1 (FOXO1), an important modulator of IGFBP-1, was further investigated using human osteosarcoma cells (U-2OS) that were transfected with pEGFP-FOXO1 and visualized by fluorescence microscopy.

Results: Glucagon significantly decreased IGF-I bioactivity in all groups ($P < 0.01$), despite unchanged total IGF-I and IGFBP-3 levels, whereas significant increases in IGFBP-1 and IGFBP-2 were observed ($P < 0.01$). In contrast to the transient increase in insulin levels in OP and LP, no change was observed in T1DM. As expected, glucagon induced a surge in GH in all subjects. In vitro, significant nuclear FOXO1 translocation was induced dose-dependently by glucagon.

Conclusion: Glucagon decreased IGF-I bioactivity in humans independently of endogenous insulin levels, likely through modulation of IGFBP-1 and-2 levels. The increased FOXO1 nuclear translocation may explain glucagon-mediated up-regulation of IGFBP-1. The glucagon-induced reduction in IGF-I bioactivity may represent a novel mechanism, underlying the impact of glucagon on GH secretion.

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Elevated level of serum leptin is associated with reduced insulin secretion in Bangladeshi impaired fasting glucose subjects

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Background and aims: Leptin is an important adipose tissue-derived hormone that has been shown to be involved in pathophysiological mechanisms related to cardiovascular disease and diabetes. However, few studies have examined the association between serum leptin and reduced insulin secretion in IFG subjects. Therefore, we explored the association of serum leptin with beta cell dysfunction in IFG subjects.

Materials and methods: Under a cross-sectional analytical design a total of 65 participants, aged 35–68 years consisting of 47.7% ($n=31$) IFG (21 male & 10 female) and 52.3% ($n=34$) healthy Controls (19 male & 15 female) were recruited for the study. Each participant underwent an oral glucose tolerance test and completed a questionnaire. Anthropometric indices were recorded and serum samples were collected for measurement. Serum leptin and insulin were measured by enzyme immunoassay. Insulin secretory function (HOMA%B) and insulin sensitivity (HOMA%S) were calculated from fasting serum glucose and fasting serum insulin values by Homeostasis Model Assessment (HOMA) using HOMA-CIGMA software.

Results: Serum leptin levels [Median (range) 16.43 (2.66–42.17) vs. 6.39 (3.03–29.54) (ng/ml), $p < 0.001$] was significantly higher in IFG group compared to the Control. HOMA%B [97 (51–163) vs. 142 (61–375), $p < 0.001$] was significantly lower in IFG group compared to the Control. On Pearson correlation analysis leptin showed significant positive correlation with gender ($r=0.417$, $p=0.019$) and significant negative correlation with age ($r=-0.545$, $p=0.002$) and HOMA%B ($r=-0.359$, $p=0.048$) in IFG subjects. In multiple linear regression analysis leptin showed significant positive association with gender ($\beta=0.396$, $p < 0.001$) and IFG group ($\beta=0.418$, $p=0.010$) however it showed significant negative association with age ($\beta=-0.157$, $p=0.022$) and HOMA%B ($\beta=-0.104$, $p=0.049$) after adjusting the major confounders (BMI and HOMA-IR). In binary logistic regression analysis age, gender, leptin and HOMA%B are independent determinants of IFG [age OR (95% CI): 1.14 (1.01–1.29), $p=0.032$, gender OR (95% CI): 0.061 (0.006–0.654), $p=0.021$, leptin OR (95% CI): 1.33 (1.13–1.58), $p=0.001$ and HOMA%B OR (95% CI): 0.93 (0.90–0.97), $p=0.001$] after adjustment of BMI.

Conclusion: Higher serum leptin levels are independently associated with insulin secretory dysfunction in IFG subjects. Thus, leptin may represent useful biomarkers for predicting the onset of glucose intolerance in Bangladeshi adults.

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The relationship between vitamin D and hypogonadism in patients with type 2 diabetes mellitusH. Dogan¹, G. Sargin², E. Harman³;¹Department of Internal Medicine, Bozyaka Training and Research Hospital, Izmir, ²Department of Internal Medicine, Canakkale State Hospital, Canakkale, ³Division of Endocrinology and Metabolism, Yesilyurt Training and Research Hospital, Izmir, Turkey.

Background and aims: Association has been reported between abnormal glucose metabolism, serum vitamin D levels and gonadal dysfunction. 25 (OH) D levels are lower in patients with Type 2 diabetic male patients compared with non-diabetic male patients. Type 2 diabetic patients were separated into three groups according to vitamin D levels (<20 ng / ml, 20–29.9 ng / ml and ≥ 30 ng / ml) and the relationship between vitamin D levels and insulin, C-peptide, fasting blood glucose, postprandial blood glucose, HOMA-IR, HbA1c, body mass index, FSH, LH, total testosterone levels were investigated.

Materials and methods: Fasting and postprandial blood glucose values were analyzed by enzymatic colorimetric method with Roche cobas 8000 brand device. HbA1c levels were analyzed by HPLC affinity boranate method with Premier Hb9210 trinity BioTec. And, insulin, C-peptide, FSH, LH, total testosterone, vitamin D levels were determined by kemiluninessans method with Beckman Coulter DXi 800. Kruskal Wallis H and Mann Whitney U tests used for statistical analysis, and <0.05 was considered as statistically significant.

Results: There is statistically significant difference between HbA1c groups and the distribution of BMI, HOMA-IR, vitamin D, fasting and postprandial blood glucose, C-peptide values ($p<0.05$). Total testosterone levels were found statistical significantly lower in the patients with vitamin D level <20 ng/ml than vitamin D level 20–29.9 ng/ml and ≥30 ng/ml (Table 1) ($p<0.05$). There is positive correlation between the level of vitamin D and total testosterone, and negative correlation between the level of FSH, LH and total testosterone. Vitamin D values were statistical significantly lower in the patients with HbA1c ≥10% than HbA1c: <6.5%, 6.5–8% and 8–10% ($p<0.05$).

Conclusion: 25 (OH) D levels should be evaluated in the patients with type 2 diabetes mellitus associated with hypogonadism. Also, it is emphasized possible detection of vitamin D deficiency and replacement simultaneously.

Table 1: Distribution of laboratory values according to Vitamin D groups

	Vitamin D <20 ng/ml	Vitamin D 20–29.9 ng/ml	Vitamin D ≥ 30 ng/ml	P ^a
HOMA-IR	5.54±8.01	4.21±4.39	5.62±8.6	0.562
FBG (mg/dl)	169.98±79.02	153.48±69.85	134.61±47.13	0.026
Insulin (uU/ml)	12.83±14.43	11.04±9.1	14.4±18.61	0.889
C-peptid (ng/ml)	3.34±2.49	2.82±1.27	3.39±1.68	0.175
PBG (mg/dl)	236.34±113.82	218.71±107.93	189.65±76.95	0.064
HbA1c (%)	7.97±2.06	7.44±1.94	7±1.12	0.017
FSH (mIU/ml)	34.07±30.92	26.43±31.68	27.65±31.28	0.224
LH (mIU/ml)	14.86±13.15	12.81±13.51	12.98±11.51	0.349
Total Testosterone (ng/ml)	1.25±1.66	2.15±1.75	2.31±1.78	<0.05
BMI (kg/m ²)	29.8±5.4	28.47±4.3	28.41±4.14	0.114

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Betatrophin, a novel potential biomarker of beta cell function in diabetesR. Lupi¹, S. Del Guerra², P. Ursoleo², C. Bianchi¹, S. Del Prato²;¹Medical Area, ²Clinical and Experimental Medicine, Metabolic Unit, Pisa, Italy.

Background and aims: Betatrophin was recently described as a potent stimulator of beta cell proliferation in mice. However, information is scanty with respect to the behavior of this hormone in metabolic disorders in human beings. Therefore, we have measured serum betatrophin levels in non-diabetic (ND), type 1 (T1DM), transplanted T1DM (TX) and type 2 (T2DM) diabetic patients. Moreover, since betatrophin is located in the corresponding intron of DOCK6, we have explored associations with the DOCK6 single nucleotide polymorphisms rs1541922 (SNP, C/T).

Materials and methods: We recruited 18 ND (49±18 yrs; 8M/10F; BMI 25.4±0.7 kg/m²), 17 T1DM (47±9 yrs; 7M/10F; BMI 24.2±1.4 kg/m²), 14 TX

(44±3 yrs; 5M/9F; BMI 21.2±2.2 kg/m²) and 33 T2DM (63±15 yrs; 18M/15F; BMI 28.5±4.7 kg/m²). Betatrophin levels were determined by an ELISA technique, C-peptide by an IRMA method, and DOCK6 SNP was genotyped using TaqMan Allelic Discrimination Assays on DNA from circulating blood cells.

Results: In the population as a whole, betatrophin levels were inversely associated with age ($p=0.0009$, $r=0.612$), triglyceride ($p=0.032$, $r=0.418$) and total cholesterol ($p=0.049$, $r=0.378$) concentrations, while a direct correlation was apparent with HDL levels ($p=0.0035$, $r=0.542$). When T1DM subjects were excluded from the analysis, betatrophin correlated with C-peptide concentration ($p=0.026$, $r=0.421$). Betatrophin was significantly higher in T1DM (892.7±84.59 pg/ml) and significantly reduced in T2DM (153.54±21.32 pg/ml) as compared to ND (440.7±46.46, pg/ml; all $p<0.05$, Bonferroni's test). In TX, betatrophin concentration returned to levels similar to those of ND (533.65±44.03 pg/ml). In the whole study population CC, CT and TT genotype frequency was 0%, 53% and 47%, respectively. Interestingly, with the exception of T2DM, the presence of the C allele in single copy was associated with a marked increase in betatrophin concentration (ND TT=268.9±11.6 and CT=386.15±47.13; T1DM TT=818.14±9.9 and CT=1043.33±89.33; TX TT=449.73±46.95 and CT=785.4±9.23; all $p<0.05$ or less).

Conclusion: The correlation between betatrophin levels and C-peptide concentrations, together with normalization of betatrophin in TX patients, supports a role of the hormone in the maintenance of beta-cell function. Whether betatrophin may provide a useful biomarker of beta cell function (or mass), however, will require further investigation. Also, a potential modulating effect of DOCK6 genotype remains to be fully explored.

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Hypothalamic-pituitary-adrenal axis dysfunction in streptozotocin(STZ)-diabetic rats: effect of lipoic acid and α-tocopherol treatmentP. Arias¹, E.M. Repetto², A.L. Bonelli², S. Rocio², M.E. Mercat², C.B. Cymeryng²;¹Chair of Human Physiology, University of Rosario Medical School,²Department of Biochemistry, University of Buenos Aires Medical School-CEFYBO-CONICET, Argentina.

Background and aims: Glucocorticoid release is involved in glucose counterregulation, and a diminished or absent adrenocortical function renders non-diabetic as well as insulin-treated diabetic subjects prone to hypoglycemia. We previously demonstrated that STZ-diabetic rats show increased basal, but markedly reduced ACTH-stimulated corticosterone (CS) levels. Present experiments, performed in STZ-diabetic rats receiving lipoic acid (LA) or α-tocopherol (aT) treatment, were designed 1) to evaluate the effect of these treatments on oxidative stress parameters, on nitric oxide synthase (NOS) activity, and on steroidogenic function of the adrenal cortex of STZ-treated rats, and 2) to test the hypothesis that STZ-induced oxidative stress might modify CS release by affecting pituitary ACTH secretion.

Materials and methods: Male Wistar rats (220–240g) were randomly assigned to the following groups: CON (control), STZ (2 injections of 40 mg/kg STZ separated by 48 h), LA (90 mg/kg ip every 48 h), aT (200 mg/kg/d po), STZ-LA or STZ-aT. LA, aT or vehicle were initiated immediately after the confirmation of hyperglycemia. After 4 weeks, all animals were sacrificed. Oxidative stress parameters and NOS activity, as well as the expression levels of CYP11A1, a key steroidogenic enzyme, and of the ACTH receptor MC2R were evaluated by RT-qPCR in the adrenal cortex. Plasma ACTH and serum CS levels (basal and ACTH-stimulated) were measured by RIA. After normality testing a one-way ANOVA was used for statistical evaluation.

Results: STZ rats displayed elevated basal CS levels, and a diminished CS response to ACTH injection. Antioxidants decreased CS levels to those observed in controls (CON: $7.3 \pm 2.5^*$, STZ: 71.7 ± 9.2 , STZ aT: $5.8 \pm 3.4^*$, STZ-LA: $19.1 \pm 5.5^*$ ng/ml; $*p<0.001$ vs STZ), and restored the defective response to ACTH. Lower basal ACTH levels were detected in STZ animals; neither LA nor aT had an impact on these changes. At the adrenal level, both drugs precluded the increase in TBARS and carbonyl content, and in the expression levels of catalase and heme oxygenase-1 detected in STZ rats. Both antioxidants also prevented the increase in NOS activity detected in diabetic animals (CON: $88.5 \pm 0.5^*$, STZ: 216.7 ± 9.2 , STZ-aT: $108.6 \pm 7.4^*$ and STZ-LA: $117.4 \pm 16.8^*$ pmol/min/mg protein; $*p<0.01$ vs STZ), and corrected the down-regulation of MC2R expression observed in the STZ group. CYP11A1 mRNA levels were not affected by any of these treatments.

Conclusion: Our results show that, in STZ-diabetic rats, generation of oxidative stress is associated with an increased activity of the adrenocortical

NO generating system, a known negative modulator of CS release. Systemic antioxidant treatment not only normalized oxidative stress parameters and NOS activity, but also corrected the observed effects of STZ-induced diabetes on CS levels. Compared to control animals, STZ-diabetic rats showed lower baseline circulating levels of ACTH, probably resulting from exaggerated basal CS output. Antioxidant therapy restored basal and ACTH-induced glucocorticoid release without modifying plasma ACTH levels, further supporting the role of local regulatory signals, such as NO, in the dysregulation of adrenal steroidogenesis observed in this animal model of diabetes.

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Progressive reduction in beta cell mitochondrial function leads to diabetes

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Background and aims: Pancreatic β -cells couple glucose stimulated insulin secretion (GSIS) with oxidative phosphorylation via the mitochondrial respiratory enzyme, cytochrome *c* oxidase (COX). Emerging evidence suggests interplay between mitochondrial dysfunction and the development of diabetes. We investigated the relation between COX activity and GSIS in isolated pancreatic islets of Cohen diabetes sensitive rats and the role it plays in diabetes development

Materials and methods: Cohen diabetic sensitive and Cohen diabetic resistant rats were maintained on a diabetogenic-diet for periods of 4, 11, 20 and 30 days. Blood glucose and insulin concentrations were measured before and during OGTT performed at different time periods on the diabetogenic-diet. Islet-COX activity was determined spectrophotometrically and normalized to citrate-synthase (a ubiquitous mitochondrial matrix enzyme).

Results: Islets of normoglycemic Cohen diabetic sensitive rats had 50% of COX activity and GSIS compared to islets of Cohen diabetic resistant rats supporting the existence of a congenital COX impairment. Following exposure to a diabetogenic-diet, Cohen diabetic sensitive rats exhibited a progressive increase in blood glucose levels and decrease in GSIS, developing overt hyperglycemia within 20 days. The decline in GSIS coincided with a significant decrease in islets-COX activity. The reduction in islet-COX activity positively correlated ($R^2 = 0.9317$) with GSIS and inversely correlated ($R^2 = 0.760$) with blood glucose levels. Cohen diabetic resistant rats maintained normoglycemia during the various time periods on diabetogenic-diet and exhibited a partial reduction in GSIS after only 30 days. Islets of Cohen diabetic resistant rats retained more than 50% of their baseline islets-COX activity after 30 days on diabetogenic-diet while in Cohen diabetic sensitive rats less than 20% of islets-COX activity remained.

Conclusion: These results show a link between islets COX activity, reduced GSIS and the development of diabetes in the Cohen diabetes sensitive rats. On the diabetogenic-diet, COX activity in islets of normoglycemic-Cohen diabetes resistant rats was reduced by 50%, while in the hyperglycemic-Cohen diabetes sensitive rats the activity was below 20%. Thus, we suggest that COX activity needs to be above a certain threshold to sustain adequate β -cell function and prevent diabetes development.

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Reduced mitochondrial biogenesis in skeletal muscle is associated with obese phenotype in androgen receptor deficient mice (ARKO)

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Background and aims: Androgen, including dehydroepiandrosterone (DHEA) and testosterone, reduces adiposity, although the underlying mechanisms are unknown. Here, we examined the effect of androgen on heat production and mitochondrial biogenesis.

Materials and methods: C57/Black male mice at 8 w of age were fed CE2 powder with (testosterone group) or without (control group) 0.4% testosterone *ad libitum* for 4wk. In addition, we assessed mitochondrial biogenesis in ARKO at 8wk and 24wk of age. ARKO was provided by Dr. Shigeaki Kato. O_2 consumption, CO_2 production and locomotor activity were measured by indirect calorimetry. The expression levels of PGC1 α , ATP5B and Cox4 protein were measured with western blot. The mRNA expression levels of PGC1 α , nuclear respiratory factor-1 (NRF-1), nuclear respiratory factor-2 (NRF-2), mitochondrial transcription factor A (Tfam) and cytochrome C, and mitochondrial DNA (mitDNA) were evaluated with real time PCR.

Results: Testosterone group exhibited weight reduction, decreased fat mass and elevated heat production without reduction of food consumption. Obese phenotype was prominent in ARKO at 24wk, but not 8wk, of age. Serum testosterone level was elevated up to approximately 12-fold, while it was decreased to 10.4% in ARKO. The expression level of PGC1 α , ATP5B and Cox4 protein in skeletal muscle, but not those in brown adipose tissue (BAT) and liver, were elevated in testosterone group, compared with control group, whereas they were suppressed in ARKO at 24wk compared with wild type littermate. Amount of mitDNA and expression level of mRNA involved mitochondrial biogenesis, such as PGC1 α , NRF-1, NRF-2, and Tfam in skeletal muscle were increased in testosterone group. Expression levels of these genes were reduced in skeletal muscle isolated from ARKO at 24wk, however significant reduction was not observed at 8wk, showing that late-onset obesity in ARKO is caused by decreased mitochondrial biogenesis in skeletal muscle. Treatment with 10 nM testosterone significantly increased mRNA levels of PGC1 α , NRF-1, NRF-2, Tfam and cytochrome C in C₂C₁₂ myotubes, which was abolished by flutamide, an AR inhibitor.

Conclusion: These results demonstrate that the testosterone-induced increase in energy expenditure is derived from elevated expression level of PGC1 α , and subsequent mitochondrial biogenesis in skeletal muscle, but not BAT. Therefore, skeletal muscle is the major organ contributing to testosterone-induced weight reduction.

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Obese but healthy: Is it really possible?

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Background and aims: Patients displaying the metabolically healthy but obese (MHO) phenotype have an intermediate cardio-metabolic prognosis as compared with normal weight healthy and metabolically unhealthy obese (MUO) patients. We aimed to evaluate the prevalence and characteristics of definite MHO individuals.

Materials and methods: Definite MHO phenotype was defined as having none of the International Diabetes Federation metabolic syndrome criteria but waist circumference criterion which is considered as non discriminatory, in 1159 patients including 943 women admitted in stable conditions for obesity (body mass index (BMI) 38.4 ± 6.3 kg/m²) without known diabetes. Patients were characterized for cardio-metabolic disorders.

Results: The 202 (17.4%) MHO individuals, younger and with lower BMI than the 957 MUO patients in the total cohort, were matched for gender, age and BMI with 404 MUO patients. In addition to the features of metabolic syndrome, the definite MHO patients as compared with the MUO ones were fewer with an homeostasis model assessment of insulin resistance index ≥ 3 (23.6 versus 38.9%, $p < 0.001$), with an abnormal oral glucose tolerance test (13.9 versus 23.9%, $p < 0.001$), HbA1c $\geq 5.7\%$ (43.9 versus 54.2%, $p < 0.05$), pulse pressure ≥ 60 mmHg ($p < 0.001$) but did not show significant differences for the prevalence of microalbuminuria (11.1 vs 12.3%), cardiac autonomic dysfunction (45.5 vs 35.3%) and fatty liver index ≥ 60 (5.6 vs 10.2%).

Conclusion: Our data do not support the characterization of MHO, even definite, as really healthy, as many patients with this phenotype have abnormal cardio-vascular markers and glucose or liver abnormalities.

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Automated quantification of brown and white adipose tissue with x-ray computer tomography (CT) and 18F-deoxyglucose positron emission tomography (FDG/PET)

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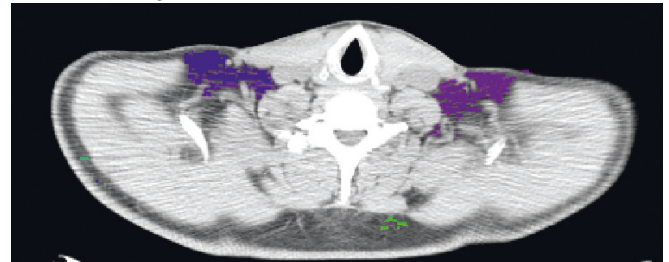
Background and aims: Brown and white, adipose tissue in the thorax differ in proportions, locations and their heat generating capability. This study uses voxel-by-voxel analysis of CT and coregistered FDG-PET to assess the volumes, activity, deposit size, and admixture of tissue type in supraclavicular, interscapular, and circumcarotid areas. This novel application of image analysis methods adapted from brain imaging is evaluated for reliability and similarity to imaging fat assessment

Materials and methods: We studied 12 insulin resistant subjects and 2 overweight healthy volunteers with FDG-PET/CT of the thorax to assess the glucose metabolic rate (GMR in $\mu\text{mol glucose}/100\text{g}/\text{min}$ and standard uptake value (SUV) of adipose tissue. Subjects were exposed to a 90-min period of cold (67°F) and warm (73°F) temperature on separate days. FDG-PET images were coregistered to a 200 mA CT. Segmented CT masks of 7 Hounsfield unit (HU) bands: -160 to -140, -140 to -120, -120 to -100, -100 to -80, -80 to -60, -60 to -40, and -40 to -20 were evaluated and compared to muscle and skin HU values. CT images had contiguous voxels automatically clustered using Analysis of Functional NeuroImages (AFNI) and the series of narrow HU unit bands as the criterion. In the -140 to -80 range, the largest supraclavicular clusters were 10–20 cm³.

Results: Greater cold minus warm differences in adipose tissue GMR than in HU defined muscle or skin were confirmed (adipose $0.06 > \text{muscle}$). Significant areas of thermogenesis in Hounsfield value defined tissue (-120 to -80) were located in 64% of subjects, a percentage similar to that obtained in visual inspection studies and a relatively broad band (-160 to -40) range of mean thermogenesis was observed. Significantly lower metabolic variation (voxel SD within the clusters) was found in supraclavicular clusters at -100 to -80 HU units and a trend observed for the variation to be higher for the cold than warm condition, suggesting a mixture of thermogenic and nonthermogenic

cells being present. Cluster size and thermogenicity were highly correlated ($r = 0.90$ to $.98$, $p < 0.001$) across the right and left thorax, consistent with reliability of the voxel clustering approach.

Conclusion: Automated algorithms based on FDG-PET/CT appear valuable in the anatomical localization, quantification, and characterization of adipose tissue temperature sensitivity. CT/FDG-PET may prove sensitive enough to detect pharmacological therapeutic interventions on different adipose tissue components. This assay complements measurements of weight, BMI, blood insulin measurement and the glucose tolerance test, as well as the sophisticated hyperinsulinemic euglycemic clamp. Fig. CT with CT-based supraclavicular clusters (right thorax cerise, left blue)



Supported by: Profil/Novartis

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Adipose tissue depot specific promoter methylation of TMEM18

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Background and aims: Epigenetic processes such as dynamic promoter methylation may play a role in obesity, fat distribution and its accompanied metabolic alterations. *TMEM18* is a candidate gene for BMI comprising the second largest effect size among all loci identified so far via GWAS. We hypothesized that differential *TMEM18* gene expression in visceral (VAT) and subcutaneous adipose tissue (SAT) may be a consequence of depot specific differential methylation at the *TMEM18* promoter region. Differential methylation levels may confer fat depot specific correlations with measures of obesity and fat distribution.

Materials and methods: (i) *TMEM18* mRNA expression was measured in VAT and SAT from 500 subjects. (ii) We investigated 146 Caucasian individuals for differential methylation levels in VAT vs. SAT at three CpG sites. (iii) Subsequently, we tested for potential correlation of methylation levels with anthropometric and metabolic parameters.

Results: (i) In 500 individuals, we observed significantly decreased mRNA expression in SAT (paired t-test, $P < 0.0001$) compared to VAT with strongest effects in obese subjects. (ii) We identified significantly higher methylation levels for the entire CpG locus in SAT (paired t-test, $P = 0.00015$). In 146 individuals, we detected positive correlations between CpG methylation levels in SAT with parameters of obesity and fat distribution (e.g. BMI, $r = 0.173$; $P = 0.036$; visceral fat area, $r = 0.246$; $P = 0.004$) and with metabolic traits ($P \leq 0.05$). However, these correlations did not withstand adjustment for covariates. (iii)

Conclusion: Our data suggest an adipose tissue depot specific *TMEM18* promoter methylation that may mediate inter-depot specific variance in *TMEM18* mRNA expression.

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A highly potent and specific PPAR alpha agonist, K-877, improves lipid profiles and insulin sensitivity in dyslipidaemia subjects; an integrated analysis of 3 Phase 2/3 trialsE. Araki¹, S. Ishibashi², S. Yamashita³, H. Arai⁴, K. Yokote⁵, H. Suganami⁶, T. Kodama⁷;¹Department of Metabolic Medicine, Kumamoto University Graduate School of Medicine, ²Division of Endocrinology and Metabolism, Jichi Medical University, Tochigi, ³Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, ⁴Department of Human Health Sciences, Kyoto University Graduate School of Medicine, ⁵Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, ⁶Clinical Data Science Department, Kowa Company, Ltd., Tokyo, ⁷Laboratory for Systems Biology and Medicine, The University of Tokyo, Japan.**Background and aims:** K-877 is a specific PPAR alpha agonist with a potentially improved pharmacological profile with respect to efficacy and safety compared with other PPAR alpha agonists. In subjects with atherogenic dyslipidemia, insulin resistance often co-exist, and as such, the treatments which concurrently improve plasma lipoprotein profiles and insulin sensitivity are highly demanded. Therefore, this integrated analysis set out to assess retrospectively the effect of K-877 on efficacy, safety and measures of insulin resistance over 12 weeks in 3 phase 2/3 trials.**Materials and methods:** This is an integrated analysis of two Phase 2 trials (one monotherapy trial and one add-on to stable pitavastatin 2 mg/day therapy) and one Phase 2/3 trial (monotherapy). All subjects had hypertriglyceridemia and low HDL-C and/or high non-HDL-C. All these were 12 weeks, randomized, multicenter, double-blinded, placebo controlled trials. The efficacy and safety of K-877 0.05, 0.1, 0.2 and 0.4 mg/day (twice daily) were examined in all trials.**Results:** A total of 676 subjects (K-877; 37, 0.05 mg/day; 127, 0.1 mg/day; 215, 0.2 mg/day; 172, 0.4 mg/day; placebo; 125) were evaluated in this integrated analysis. The subject baseline characteristics were comparable among all groups. From baseline to Week12, a significant reduction in TG and an increase in HDL-C were observed for K-877 groups (TG; -40.6 to -52.7%, HDL-C; +12.0 to +24.0%, $p < 0.001$ for all). In K-877 groups, the effects on TG and HDL-C were similar regardless of baseline HOMA-IR. Favorable effects on insulin sensitivity were also observed dose-dependently in K-877 groups [fasting plasma glucose (FPG): K-877; +4.62 to -5.53 mg/dL, placebo; +2.95 mg/dL, fasting plasma insulin (FPI): K-877; -0.87 to -3.36 μ U/mL, placebo; -0.28 μ U/mL, HOMA-IR: K-877; -0.35 to -1.23, placebo; +0.01]. The reduction of FPG, FPI and HOMA-IR was greater in the subjects with higher HOMA-IR. The incidence of all adverse drug reactions in K-877 groups were similar to placebo (placebo; 8.0%, 0.05mg/day; 5.4%, 0.1mg/day; 4.7%, 0.2mg/day; 7.9%, 0.4mg/day; 8.1%). Similar results were observed in the incidence of adverse events including abnormal liver function test, increased blood creatine phosphokinase and increased blood creatinine.**Conclusion:** In an integrated analysis, K-877 improved lipid profiles and insulin sensitivity without increasing adverse events. K-877 could be a promising therapy for atherogenic dyslipidemia. The mechanisms how K-877 improve insulin sensitivity should need further investigation.

Clinical Trial Registration Number: JapicCTI-121837(ja), JapicCTI-121764(ja), JapicCTI-121837(ja)

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Genomic profiling in plasma reveals the effect of a polyunsaturated fatty acid-enriched diet on common microRNAsJ.M. Mercader¹, F.J. Ortega², M.I. Cardona-Alvarado³, J.M. Moreno-Navarrete², M. Moreno², M. Sabater², N. Fuentes-Batllevell², W. Ricart², E.L. Pérez-Luque², J.M. Fernández-Real²;¹Joint IRB-BSC program on Computational Biology, Barcelona, ²Department of Diabetes, Endocrinology and Nutrition (UDEN), Institut d'Investigació Biomèdica de Girona (IdIBGi), Girona, Spain, ³Department of Medical Research, Division of Health Sciences, Leon Campus, Mexico.**Background and aims:** Consumption of long-chain polyunsaturated fatty acids (PUFAs) of omega n-3 and n-6 series, which are abundant in seafood and nuts, ameliorate components of the metabolic syndrome, reducing LDL-cholesterol. Mechanisms responsible for diet-induced LDL reduction are likely associated with nutrients provided by these aliments, targeting primary

routes of LDL reduction and regulating enzymes involved in cholesterol synthesis. Circulating microRNAs (miRNAs) have demonstrated to be valuable biomarkers of metabolic diseases. Here, we investigated whether a sustained nuts-enriched diet can lead to changes in circulating miRNAs in parallel to LDL-cholesterol reduction.

Materials and methods: The profile of 192 common miRNAs was assessed in plasma from 10 healthy women before and after an 8-week trial with a normocaloric diet enriched in PUFAs (30 g/day of almonds and walnuts). The most relevant miRNAs were validated in an extended sample of 30 participants (8 men and 22 women).**Results:** Concomitantly with decreased body mass index (-3.5%, $p < 0.0001$), circulating LDL (-10%, $p < 0.001$), and increased adiponectin (35%, $p = 0.017$), circulating concentrations of several miRNAs were modified by treatment, including decreased miR-330-3p, miR-328 and miR-221, and increased miR-769-5p, miR-18a, miR-130b, miR-192, miR-19b, and miR-486-5p (all $p < 0.05$). Interestingly, miR-130b ($r = 0.7$, $p < 0.0001$), miR-221 ($r = 0.44$, $p = 0.03$), and miR-409 ($r = 0.6$, $p = 0.001$) variations correlated with changes in circulating C-reactive protein concentrations, while miR-328 ($r = 0.5$, $p = 0.01$) and miR-125b ($r = 0.63$, $p = 0.002$) were associated with adiponectin. Noteworthy, diet-induced changes in circulating miR-486-5p were also associated with dietary fatty acids intake ($r = 0.44$, $p = 0.02$), miR-221 variations were inversely associated with increased dietary omega n-3 ($r = -0.5$, $p = 0.01$), and modifications on circulating miR-192 correlated with dietary omega n-6 ($r = 0.62$, $p = 0.001$).**Conclusion:** This study provides the first evidence that circulating miRNAs may be modified by polyunsaturated fatty acid-enriched diet and reveals their potential relevance in this context.

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Lysophosphatidylcholines as regulators of gene expression in human skeletal muscleC. Klingler¹, M. Wolf¹, J. Li², S. Chen², X. Zhao², E. Schleicher^{3,4}, H.-U. Häring^{3,4}, G. Xu², R. Lehmann^{3,4}, C. Weigert^{3,4};¹Department of Internal Medicine IV, University Tuebingen, Germany, ²Dalian Institute of Chemical Physics, Dalian, China, ³German Center for Diabetes Research, Tuebingen, ⁴Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tuebingen, Germany.**Background and aims:** Lysophosphatidylcholines (LPC) circulating in the blood stream recently gained attention as biomarkers for insulin resistance and T2DM. It remains to be resolved whether LPC plasma concentrations can contribute to the development of insulin resistance. Skeletal muscle might represent not only a target for LPC but might be a source of circulating LPC.**Materials and methods:** Human Myocytes from biopsies of healthy young volunteers were differentiated to myotubes in AlphaMEM with 2% FBS for 5 days. Myotubes were stimulated for 24 h, 4 h, and 30 min with ¹³C-labelled palmitate (0.250 mM). Afterwards LPC biosynthesis and secretion was quantified by isotope-based LPC profiling. Furthermore, myotubes were stimulated with different concentrations of LPC C16:0 and LPC C18:1 followed by transcriptomics analysis, qPCR, siRNA-based knock-down und gel shift assays.**Results:** In primary human myotubes stimulation with palmitate resulted in the synthesis of several LPC species leading to time-dependent intracellular accumulation of LPCs. Moreover newly synthesized LPCs were released into the extracellular compartment. After stimulation of myotubes with 10 μ M LPC (16:0) or LPC (18:1) whole genome expression analysis revealed an activation of PPAR δ -regulated transcripts. qPCR confirmed the induction of the PPAR δ target genes PDK4 and ANGPTL4 mRNA already at a LPC concentration of 1 μ M. This induction was attenuated in the presence of siRNA against PPAR δ . By gel shift assays we could demonstrate that LPCs regulate DNA binding activity of the PPAR δ /RXR α complex in a dose-dependent manner.**Conclusion:** Extracellular LPCs can activate PPAR δ -dependent gene expression in human myotubes as direct ligands of this nuclear receptor. LPCs originating from muscle could act in a paracrine or endocrine fashion on lipid metabolism of different target tissues.

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Lower intensified target LDL-C level of statin therapy results in a higher risk of incident diabetes: a meta-analysis of randomised statin trials

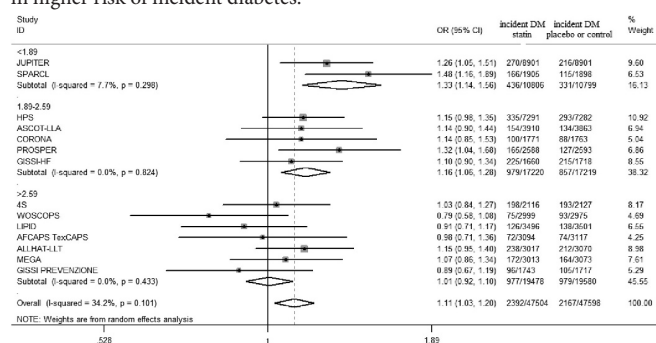
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Background and aims: A recent meta-analysis has reported that intensive-dose statin drug increases the risk of incident diabetes. However, doubling of the statin dose generates only a further 6% decrease in low-density lipoprotein cholesterol (LDL-c) on average. This study aimed to determine whether statin therapy with lower intensive-target LDL-c level contributes to higher risk of new-onset diabetes.

Materials and methods: Medline, Embase, and the Cochrane Central Register of Controlled Trials were searched for randomized controlled endpoint trials of statins conducted from 1966 to 2012. We included trials with more than 1000 participants who were followed up for at least 2 years. The included trials were stratified by the target LDL-c level. I^2 statistic was used to measure heterogeneity between trials. We further calculated risk estimates with random-effect meta-analysis. Meta-regression was used to identify the potential risk factors of statin-induced diabetes.

Results: Fourteen trials with a total of 95 102 non-diabetic participants were included. The risks elevated by 33% [odds ratio (OR) = 1.33; 95% confidence interval (CI) 1.14–1.56; $I^2 = 7.7\%$] and 16% (OR = 1.16; 95% CI 1.06–1.28; $I^2 = 0.0\%$) when the intensified target LDL-c levels were ≤ 1.8 mmol/L and 1.8–2.59 mmol/L, respectively. Incident diabetes did not increase when the target LDL-c level was ≥ 2.59 mmol/L. Apart from age, female, and baseline level of total cholesterol, meta-regression analysis showed that the target and baseline levels of LDL-c and relative LDL-c reduction were predictors of statin-induced diabetes.

Conclusion: A lower intensified target LDL-c level of statin therapy resulted in higher risk of incident diabetes.



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Intensified LDL-C target of statin therapy and cancer risk: a meta-analysis

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Background and aims: Statin therapy and cancer incidence have no relationship according to a recent system review and meta-analysis. However, whether an intensified low-density lipoprotein-cholesterol (LDL-c) target of statin therapy is associated with cancer incidence has been rarely reported.

Materials and methods: PUBMED, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) data as of July 2013 were searched for randomized controlled trials (RCTs) on statins. An intensified LDL-c target of <2.59 mmol/L (100 mg/dl) or a relative LDL-c reduction by at least 30% of the baseline was the primary criterion for all the trials that were included in the meta-analysis. The I^2 statistic was used to measure heterogeneity among the trials, and risk estimates were calculated for incident cancer with random-effect meta-analysis.

Results: Eleven eligible studies were identified with 70,714 participants, 6,423 of whom (3,231 were given statins and 3,192 were given control treatment) developed cancer during the follow-up period. The intensified LDL-c target of statin therapy showed no effect on cancer incidence (odds ratio [OR] 1.02,

95% CI 0.94–1.11, $I^2=38.2\%$), including some common types of respiratory and gastrointestinal cancer. However, pravastatin seemed to promote cancer incidence (OR 1.26, 95% CI 1.04–1.53). Meta-regression analysis showed that neither the chemical properties nor the species of the statins accounted for the residual variation in risk.

Conclusion: The intensified LDL-c target of statin therapy has no effect on the overall incidence of cancer, including some common types of respiratory and gastrointestinal cancer. Thus, such an intensified statin therapy need not be changed among adult clinical patients.

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Age at menarche, glycaemic control, cardiovascular risk factors and chronic complications in women with type 1 diabetes: a nationwide survey in Brazil

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Background and aims: Puberty is an early life event that may have several relations to later life diseases risk and is an event that is usually well recalled by adult women being a reliable measure of pubertal initiation. Earlier age at menarche onset has been associated with several adverse outcomes, such as type 2 diabetes, obesity, cardiovascular diseases, metabolic syndrome and all causes mortality, showing that the timing of pubertal development may have lifelong effects on a woman's health. So far many menstrual irregularities were described in women with type 1 diabetes like early and delayed menarche timing, menstrual cycle irregularities and early menopause. In general a higher age at menarche is observed in women with type 1 diabetes in comparison to controls of the same population, but this difference is not significant. The influence of glycemic control upon age at menarche is still controversial. The aim of this study was to determine the relationship between age at menarche with glycemic control and cardiovascular risk factors in patients with type 1 diabetes in Brazil.

Materials and methods: This was a multicenter cross-sectional study conducted between December 2008 and December 2010 in 28 public clinics in 20 cities from the four Brazilian geographic regions. Data were obtained from 1,527 female patients, 59.3% Caucasians, aged 25.1 ± 10.6 years. The diabetes duration was 11.4 ± 8.1 years. Patient information (clinical factors and age at menarche) was obtained through a questionnaire and a chart review. Age at menarche was stratified in four classes: 8 to 11 (class 1, early menarche), 12 (class 2), 13 (class 3) and 14–18 years (class 4, late menarche).

Results: The mean age at menarche was 12.7 ± 1.7 years (median 13.0 years) without difference between the geographical regions of the country, economic status, level of care and ethnicity. Patients from class 1 had greater BMI than patients from the other classes, $p<0.001$, and were more likely to be overweight or obese than patients from the other classes, [169 (31.5%) vs 152 (28.3%), vs 117 (21.8%) vs 99 (18.4%), $p<0.001$], respectively. BMI had an inverse correlation with age at menarche ($r=-0.14$, $p<0.001$). No significant difference was observed among the four classes of age at menarche for blood pressure, lipid profile and diabetes-related chronic complications. More patients from class 1 were using metformin in comparison to patients from class 4, 13.8% vs 7.1%, $p=0.002$, respectively. Logistic regression analysis showed that early age at menarche, [8–11 years, (odds ratio (ORs) 1.77 [1.30–2.41, $p<0.001$]] and duration of diabetes [ORs 1.01 (1.00–1.03), $p=0.02$], were related to greater risk of patients' overweight or obesity and adherence to diet [ORs 0.78 (0.60–0.93), $p=0.01$], physical activity [ORs 0.75 (0.94–0.94), $p=0.01$ insulin dose (U/kg) (ORs 0.54 [0.59–0.90, $p=0.001$]] were related to lower risk for overweight or obesity.

Conclusion: Early menarche occurred in 23.4% of Brazilian female with type 1 diabetes and was strongly associated with overweight or obesity in pubertal/adult life which could be minimized with lifestyle changes such as better adherence to diet and physical activity. Further prospective studies must be addressed to establish the relationship between early menarche, glycemic control, cardiovascular risk factors and diabetes-related chronic complications.

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PS 046 Brain and glucose metabolism

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MAPK signalling is required for brain insulin to suppress lipolysis in rodents

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Background and aims: Insulin is the major anti-lipolytic hormone and is secreted postprandially. We recently showed that brain insulin action is able to suppress lipolysis by reducing sympathetic nervous system outflow to white adipose tissue. However, the relative importance of brain insulin versus the well-characterized peripheral insulin effects in regulating whole body lipolysis is unknown.

Materials and methods: Here, we studied the role of brain insulin versus peripheral insulin action using two inducible insulin receptor knock-out mouse models and their respective controls. The first model lacks the insulin receptor (IR) in the whole body (IRdeltaWB), while the second model lacks the IR in all peripheral tissues (IRdeltaPER) except the brain. All mice were subjected to 2-hour hyperinsulinemic euglycemic clamp studies with stable isotope glycerol tracer to assess whole body lipolysis. To further identify the signal transduction pathway mediating brain insulin's effects on lipolysis, we co-infused insulin with either the PI3K inhibitor LY294002 or the MAPK inhibitor U0126 directly into the mediobasal hypothalamus (MBH) of male Sprague Dawley rats ($n \geq 4$ per group) using stereotactically implanted cannulae, and assessed lipolytic flux using the rate of appearance (Ra) of the glycerol tracer.

Results: Male IRdeltaPER knock-out mice subjected to a hyperinsulinemic clamp showed a comparable % suppression of Ra glycerol per unit of insulin (4.7 ± 1.7 vs. 6.0 ± 1.4 %; $P=0.6$) to littermate controls despite being extremely insulin resistant (glucose infusion rate 21 ± 5 vs. 97 ± 10 mg*kg⁻¹*min⁻¹; $P=0.001$). In contrast, male IRdeltaWB knock-out mice were unable to adequately suppress lipolytic flux (0.4 ± 0.2 vs. 6.9 ± 2.2 %, $P=0.017$) compared to control mice when exposed to high doses of insulin during a clamp. MBH insulin co-infusion with LY294002 suppressed Ra glycerol to a similar extent than MBH insulin alone (17 ± 2 vs. 15 ± 6 vs. 32 ± 7 (controls) $\mu\text{mol*kg}^{-1}\text{*min}^{-1}$; $P=0.7$ MBH LY+Insulin vs. MBH Insulin) in rats. However, the co-infusion of the MAPK inhibitor U0126 blocked the ability of insulin to suppress lipolytic flux (60 ± 8 $\mu\text{mol*kg}^{-1}\text{*min}^{-1}$; $P=0.006$ vs. MBH LY+Insulin; $P<0.001$ vs. MBH Insulin).

Conclusion: Insulin suppresses lipolysis even after the complete loss of peripheral insulin receptors, provided that functional brain insulin receptors and an intact MAPK pathway are present. Conditions in which brain insulin signaling is impaired, such as high fat feeding and obesity, are likely to compromise the regulation of lipolysis and lead to uncontrolled free fatty acid flux promoting lipotoxicity.

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Alteration of hypothalamic glucose sensing in high fat-high sucrose diet fed rats: early defects linked to mitochondrial dynamics and mROS signalling

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Background and aims: The hypothalamus participates in the control of energy homeostasis by detecting circulating nutrients, such as glucose. The mediobasal hypothalamus (MBH), in particular, senses hyperglycemia and initiates physiological responses, e.g., insulin secretion (IS) via the autonomous (vagal) nervous system. We have recently demonstrated that glucose sensing (GS) requires mitochondrial reactive oxygen species (mROS) signaling heavily dependant on mitochondrial fusion and fission (dynamics). Recently, genetic models have associated some of these dynamics within the MBH to their obesogenic susceptibility. Using a model that only presents a hypothalamic GS defect, our objectives were thus to determine whether modulating the diet affects 1) mROS signaling, 2) mitochondrial fission and fusion, 3)

respiratory function in the hypothalamus, and how early these defects could be detected during the progression to diabetes.

Materials and methods: Male Wistar rats were fed a High Fat-High Sucrose (HFHS) diet during 3 weeks and compared to control rats fed a normal chow. Circulating hormones and metabolites were evaluated as well as glucose tolerance. Hypothalamic glucose-induced IS was studied in response to a carotid glucose injection towards the brain without alteration in peripheral glycemia and compared to islet glucose-induced IS. Hypothalamic ROS production and mitochondrial dynamics (main actors: DRP1 (fission), MFN2(fusion)) were quantified. Efficiency of hypothalamic mitochondrial respiration was evaluated using oxygraphy, as well as the redox status (glutathion red/ox) of MBH explants.

Results: A 3 weeks HFHS diet did not significantly alter body weight, despite the caloric intake ($\text{STD}=72.3 \pm 1.2 \text{ kcal/j}$; $\text{HFHS}=83.9 \pm 1.8 \text{ kcal/j}$; $p<0.001$) and the fat mass ($\text{STD}=11.8 \pm 0.6\%$; $\text{HFHS}=15.1 \pm 0.8\%$; $p=0.007$) being increased compared to control rats. HFHS fed rats displayed a glucose intolerance in the first 30 min, an increased fasting glycemia but no modification of fasting insulinemia. Hypothalamic GS induced IS was drastically decreased (Δ insulin: $\text{STD}=86.1 \pm 26.9 \mu\text{U/ml}$; $\text{HFHS}=26.9 \pm 11.4 \mu\text{U/ml}$; $p=0.017$) while glucose stimulated IS in isolated islets was not different compared to controls. These defects correlate with a decrease of MBH ROS production (-33%). The fission protein DRP1 exhibited a decreased mitochondrial translocation in the MBH, and no change in MFN2 (fusion) content was observed while controls exhibit an increase. Finally, mitochondrial respiratory deficiencies were present in HFHS fed rats.

Conclusion: A hypothalamic alteration of mitochondrial ROS signaling, fission and fusion, and respiration was present in rats exposed to a 3 weeks HFHS diet. Such hypothalamic GS defects are early events preceding those in islets. These results suggest that the glucose intolerance seen in the first 30 min involved the hypothalamo-pancreatic axis rather than the islet GS (the vagal control being of particular importance for this first phase). In conclusion, these early but drastic hypothalamic modifications could participate in a primary nervous defect of the control of insulin secretion, and finally, the establishment of a diabetic phenotype.

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Brain glutamate dehydrogenase (GDH) knockout mice display modified central glucose metabolism reshaping peripheral energy distribution

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Background and aims: Glucose is the prominent source of energy delivered to the central nervous system (CNS) by the periphery. However, within the brain, oxidative catabolism of the main neurotransmitter glutamate contributes to maintenance of energy homeostasis. Upon glutamatergic transmission, following its uptake by astrocytes, glutamate may be amidated to glutamine and then recycled back to neurons. Alternatively, glutamate may be deaminated to alpha-ketoglutarate by the mitochondrial enzyme glutamate dehydrogenase (GDH, encoded by the Glud1 gene) before further oxidation in the TCA cycle, ultimately producing ATP. We generated brain-specific GDH knockout mice (CnsGlud1^{-/-}) to question the importance of GDH as a key enzyme connecting glucose and glutamate metabolism within the CNS.

Materials and methods: Mitochondrial oxygen consumption and cytosolic ATP levels were measured in isolated brain tissues and primary astrocytes. Glucose uptake was estimated by incorporation of injected 2-deoxy-D-[14C] glucose. Brain metabolites were measured by NMR in living animals otherwise using commercial kits. Peripheral glucose homeostasis was assessed by ip glucose tolerance tests. Insulin resistance was assessed by hyperinsulinemic-euglycemic clamps.

Results: In CnsGlud1^{-/-} brains lacking GDH, glutamate oxidation was reduced, resulting in elevated glutamate plus glutamine pool and impaired glutamate-induced oxygen consumption and ATP generation in their astrocytes. CnsGlud1^{-/-} mice compensated for the lack of glutamate usage by increasing brain glucose consumption. This adaptive mechanism was uncovered by the observed lower central glucose concentrations (-47%, $p<0.05$), enhanced brain glucose uptake (+32%, $p<0.05$) and increased glucose-stimulated ATP generation in their astrocytes. Higher brain glucose consumption in Cns-

Glut1^{-/-} mice was confirmed by measurements of elevated incorporation of ¹³C from [U-¹³C]-glucose into those amino acids representative for oxidative metabolism of glucose products in the TCA cycle (alanine, aspartate, glutamate). CnsGlut1^{-/-} mice were glucose intolerant with normal insulin production. However, they displayed increased endogenous glucose production and decreased muscle glucose uptake. Fat turnover was also modified with higher circulating triglycerides and ketone bodies. Furthermore, plasma concentrations of most amino acids were lower in CnsGlut1^{-/-} mice in comparison with control mice, suggesting increased mobilization of metabolites in CnsGlut1^{-/-} skeletal muscles for provision of brain-specific energy substrates. Lower plasma pancreatic polypeptide and higher catecholamines levels highlighted repressed parasympathetic activity with a concomitant increase in the sympathetic input favouring energy mobilization.

Conclusion: Brain-specific GDH deletion induced impairment of glutamate oxidation in the CNS leading to central hypoglycaemia; in turn mobilizing energy substrates from the periphery.

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Acyl ghrelin acts in the brain to control liver function and peripheral glucose homeostasis

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Background and aims: The gut hormone, ghrelin, acts on neuropeptide Y neurons in the arcuate nucleus of the hypothalamus and stimulates food intake to control energy balance. However, recent evidence suggests that peripheral ghrelin regulates glucose metabolism. Here, we designed experiments to examine how central acyl ghrelin infusion affects peripheral glucose metabolism under pairfed or ad libitum feeding conditions.

Materials and methods: Three groups of mice received either ICV infusion of aCSF, ghrelin and allowed to eat ad libitum (Gh-lib) or ghrelin and pairfed to the average of the aCSF group (Gh-pf). Mini pumps delivered acyl ghrelin at a dose of 0.25 µg/hour at 0.5 µl/hour for 7 days.

Results: There was no difference in daily fed blood glucose, insulin, glucagon, triglycerides or non-esterified fatty acids between the groups. Body weight gain and food intake was significantly higher in Gh-lib mice compared to aCSF and Gh-pf. However, both Gh-lib and Gh-pf groups exhibited heavier white adipose mass, independent of body weight and food intake. Gh-pf mice exhibited a state of negative energy balance, as hypothalamic NPY and AgRP mRNA expression was increased in Gh-pf mice relative to aCSF or Gh-lib. Gh-pf mice exhibited better glucose tolerance than aCSF or Gh-lib mice during a GTT, although both Gh-lib and Gh-pf increased insulin release during the GTT. Central acyl ghrelin infusion and pairfeeding also increased breakdown of liver glycogen and triglyceride, and regulated genes involved in hepatic lipid and glucose metabolism. Gh-pf mice had an increase in plasma blood glucose during a pyruvate tolerance test in relative to Gh-lib or aCSF mice. Our results suggest that under conditions of negative energy (Gh-pf mice) central acyl ghrelin engages a neural circuit that influences hepatic glucose function.

Conclusion: Metabolic status affects the ability of central acyl ghrelin to regulate peripheral glucose homeostasis.

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Increasing central exposure of an MC4 agonist potentiates food intake and body weight lowering transiently in lean mice

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Background and aims: It is well known that Melanocortin-4 receptors (MC4r) are widely expressed inside (hypothalamus) and outside (circumventricular organs, brain stem) the blood brain barrier (BBB) and that hypothalamus play a central role in regulation of food intake (FI) and body weight (BW). Interestingly, MC4r agonistic peptides which do not penetrate the BBB, decrease FI and BW in rodents both after peripheral and central

administration. This study investigated whether increased CNS penetration potentiates the effects on FI and BW lowering of an orally administered small molecule MC4 agonist, cpd X.

Materials and methods: Female wildtype (WT) and Mdr1a/b-Bcrp triple targeted mutation (KO) mice on FVB background were dosed orally twice daily for 10 days with vehicle, or cpd X at 0.5 µmol/kg (only KO) or 15 µmol/kg. Individual FI and BW were recorded daily, including 2 base line days, while plasma and brain PK profiling were performed after 1 and 10 days of dosing. Lean and diet induced obese (DIO) mice on C57Bl/6 background were dosed twice daily with either vehicle or 30 µmol/kg cpd X. Analysis of Covariance, and 5% significance level tests based on Student's t-test were used for statistical validation (n=5-8).

Results: Cpd X dosed at 15 µmol/kg resulted in plasma exposure (AUC_{0-24h}, day 1) of 5.7 and 3.3 h*µmol/L in KO and WT mice, respectively, while the unbound brain/plasma ratio was more than 40 times higher in KO compared to WT mice. Cpd X significantly reduced FI and BW over the first 24hr of treatment in WT FVB (15 µmol/kg; FI: -20.2 ± 6.9%, BW: -2.3 ± 1.2%) and KO mice (FI: 0.5 µmol/kg -21.7 ± 6.3% and 15 µmol/kg -48.5 ± 8.9%, BW: 0.5 µmol/kg -0.9 ± 0.8% and 15 µmol/kg -5.1 ± 1.0%) relative to vehicle. The effect on FI was transient and returned towards vehicle levels after 48 hours especially in the KO mice. In contrast, BW lowering was sustained for the remainder of the study, especially for the WT, but also for the high dose KO mice. Cpd X (30 µmol/kg) reduced BW 10.3 ± 1.7% in DIO mice but had no effect in lean C57Bl/6 mice after 10 days treatment.

Conclusion: Higher brain exposure of cpd X was associated with greater transient reductions in FI and BW over the first 24 hours only. The initial effects support the hypothesis that CNS penetration of MC4r agonists potentiates FI/BW lowering. The lack of durable efficacy may be due to the FVB genetic background or the fact that the mice used were not obese. The latter is supported by the lack of BW reduction in lean versus DIO mice on C57Bl/6 background. Thus, lean FVB mice are unlikely to be predictive for the long-term effects on FI/BW lowering in C57Bl/6 DIO mice.

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Lack of alpha-MSH in POMC neurons controls hepatic glucose production

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Background and aims: Anorexigenic proopiomelanocortin (POMC)-expressing neurons in the arcuate nucleus of the hypothalamus constitute a fundamental nexus in the neuronal hierarchy controlling whole-body energy balance. In particular, POMC neurons are able to sense and integrate a range of nutrient and hormonal cues informing about the energy status of the organism. Mitochondrial fusion, the merge of two mitochondria into one single compartment, is a key process implicated in mitochondria quality control and also in the bioenergetic adaptations to changes in environmental nutrient availability. A key protein mediating this fusion processes is Mitofusin 2 (Mfn2). We tested the hypothesis that Mfn2 in POMC neurons may be an important component of the sensing machinery implicated in the regulation of systemic glucose homeostasis.

Materials and methods: Mice lacking Mfn2 in POMC neurons (POMC-Mfn2KO) were generated and detailed phenotyping performed.

Results: Young 6-week old POMC-Mfn2KO mice showed unaltered body weight and adiposity when compared to control counterparts. However, they exhibited mild glucose intolerance and insulin resistance. In vivo glucose-stimulated insulin release and pancreatic morphometric parameters were unaltered in POMC-Mfn2KO mice. Mutant mice showed increased glucose levels in response to pyruvate test and upregulation of key hepatic gluconeogenic genes, enzymatic activity and proteins, consistent with enhanced hepatic glucose production (HGP). POMC-Mfn2KO mice also showed reduced insulin signaling in the liver indicating insulin resistance. These alterations were related to a dramatic reduction in alpha melanocyte stimulating hormone (αMSH), the main anorexigenic peptide released by POMC neurons.

ICV α MSH administration was able to reverse most of the glucose metabolism alterations observed in POMC Mfn2KO mice.

Conclusion: Deletion of Mfn2 in POMC neurons leads to defective glucose homeostasis independent of alterations in body weight. Our data indicate that the underlying cause is reduced α MSH production that eventually leads to enhanced HGP.

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Impaired cognition coexists with hippocampal remodelling obstacles in LDL receptor knock-out mice

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Background and aims: Evidences from clinical studies support that the abnormal cholesterol metabolism in the brain lead to the progress of cognitive dysfunction. Low-density lipoprotein receptor (LDLR) is well known for its role in regulating brain cholesterol homeostasis. We investigated whether LDLR plays roles in cognition and the related potential mechanisms.

Materials and methods: Twelve-month-old $\text{Ldlr}^{-/-}$ mice ($n=12$) and wild-type littermates C57BL/6J ($n=14$) maintained on a standard lab chow diet were subjected to the morris water maze test, sucrose consumption test, and open-field test when they were 4, 7, and 12 month-old, respectively. All animals were killed for the hippocampal remodeling studies, including the hippocampal neural stem cells (NSCs) proliferation, survival and differentiation studies, as well as synapse and apoptosis studies one week after the completion of all behavioral tests.

Results: The plasma cholesterol concentrations of $\text{Ldlr}^{-/-}$ mice increased moderately than C57BL/6J ($P<0.05$). The results of behavioral tests revealed that $\text{Ldlr}^{-/-}$ mice displayed impaired spatial memory, increased anhedonia and hyperactivity (all $P<0.05$), with decreased hippocampal BrdU^+ cells ($P<0.01$), $\text{BrdU}^+/\text{NeuN}^+$ and BrdU^+ cells ratio ($P<0.05$), and reduced expression levels of synaptophysin as well as number of synaptophysin-immunoreactive presynaptic boutons in the hippocampal CA_1 and dentate gyrus areas (all $P<0.05$). Ultrastructural changes in DG area of the hippocampus were observed by transmission electron microscopy. Furthermore, the apoptosis occurred in the hippocampus of $\text{Ldlr}^{-/-}$ mice was discovered with the elevated Bax/Bcl-2 expression ratio at both mRNA and protein levels ($P<0.05$; $P<0.01$, respectively), and increased activated-caspase3 level ($P<0.05$).

Conclusion: Deteriorations of brain cholesterol homeostasis induced by LDLR deficiency contributes to impaired spatial cognition, increased anhedonia and hyperactivity, probably via its negative effects on hippocampal remodeling, including reduced hippocampal NSCs proliferation, survival and differentiation as well as increased hippocampal vulnerability to apoptosis and synapse deficits.

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Poorly controlled cholesterol is associated with cognitive impairment in type 2 diabetic patients: a resting state fMRI study

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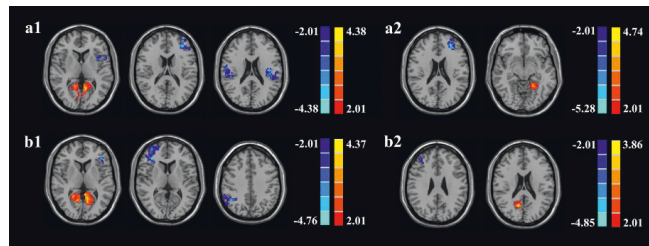
Background and aims: Debate remains on whether hypercholesterolemia, which often exist in T2DM patients, is etiologically associated with cognitive impairment or dementia. This study aims to investigate whether poor controlled cholesterol impaired functional connectivity among patients with type 2 diabetes mellitus (T2DM).

Materials and methods: We used resting-state functional magnetic resonance imaging (fMRI) to investigate the functional connectivity of 25 T2DM patients with poor controlled cholesterol, 22 patients with target cholesterol and 26 well-matched healthy controls. Further correlation analysis was conducted between functional connectivity and clinical data as well as neuropsychological tests.

Results: The three groups did not statistically differ in age, sex, education level, BMI, blood pressure, fasting C-peptides, and triglyceride. Compared with target cholesterol patients, patients with poor controlled cholesterol had significantly higher serum cholesterol levels, LDL, LDL/HDL index and worse performance in TMT-B ($p < 0.05$). Apart from these, no other significant

differences were noted between the two groups. Disordered functional connectivity of bilateral hippocampus-middle frontal gyrus (MFG) in the poor controlled group had been consistently shown when compared with the other two groups. Besides, the aberrant functional connectivity was associated with TMT-B scores as well as the LDL/HDL index in T2DM patients with poor controlled cholesterol.

Conclusion: T2DM patients with poor controlled cholesterol show impaired attention and executive function. Resting-state connectivity disturbance of the hippocampus-MFG may be involved in this process. Lowering the LDL/HDL ratio might be taken precaution against cognitive decrements.



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Impact of insulin resistance on memory performance, brain morphology and neurochemical profiles of the cortex and hippocampus

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Background and aims: Insulin signalling is involved in metabolic regulation and synaptic plasticity in the central nervous system. Glucose neurotoxicity and reduced insulin signalling may impair brain structure and function leading to behavioural and cognitive alterations. In the present study we aimed at identifying brain metabolic modifications upon altered insulin signalling, underlying or leading to neurodegeneration and eventual structural and functional anomalies.

Materials and methods: Hippocampal-dependent memory of Wistar rats ($n=12$) and insulin-resistant Goto-Kakizaki rats (GK, $n=8$) was evaluated by measuring spontaneous alternation in a Y-maze at 2, 4 and 6 months of age. After behavioural testing at each age, magnetic resonance imaging (MRI) and spectroscopy (MRS) were performed under 2% isoflurane anaesthesia in oxygen gas, on a 14.1 T spectrometer using a home-built quadrature surface coil. MRI was performed with fast-spin-echo imaging with 4 s repetition time (TR) and 52 ms echo time (TE) and images were analysed with ImageJ. MRS was performed in hippocampal and cortical areas using Spin Echo, full Intensity Acquired Localized spectroscopy with TE=2.8 ms and TR=4 s. Spectra were analysed with LCmodel to quantify 20 metabolites composing the neurochemical profile. A glucose tolerance test (GTT) was performed by glucose administration (i.p. 2 g/kg) after fasting 18 hours.

Results: Fasting glycaemia was 3.4 ± 0.1 and 4.3 ± 0.2 mmol/L, while serum insulin was 4.0 ± 1.1 and 5.9 ± 1.6 mU/L, for Wistar and GK rats respectively. This resulted in a fasting glucose to insulin ratio of 4.8 ± 1.8 for Wistar and 1.0 ± 0.2 mol/U for GK rats, depicting a 79% reduction in insulin sensitivity. The GTT area under the curve was 11.6 ± 1.7 and 42.3 ± 3.4 mmol.h/L for Wistar and GK rats, respectively. At all tested ages, GK rats displayed impaired memory performance, as depicted by reduced Y-maze spontaneous alternation (-14% to -22% relative to controls, $P<0.01$). The brain of GK rats displayed smaller hippocampal volume (-18% to -20% of controls, $P<0.001$) and larger volume of lateral and third ventricles (+39% to +84% of controls, $P<0.001$). Interestingly, glucose intolerance correlated with hippocampal ($r=-0.86$, $P<0.001$) and ventricular ($r=0.57$, $P<0.01$) volumes. Hippocampal volume further correlated with memory performance ($r=0.47$, $P<0.001$). The neurochemical profiles were affected by insulin resistance. Most notably, in comparison to controls, GK rats displayed reduced glutamine (-14% to -24%, $P<0.001$) and choline (-13% to -21%, $P<0.001$) in both regions. In the hippocampus, GK rats had higher taurine (+8% to +16%, $P<0.001$) and ascorbate (+38% to 48%, $P<0.001$) and reduced alanine (-19% to -30%, $P<0.01$) concentrations relative to controls. Cortical aspartate content was lower in GK relative to controls (-14% to 29%, $P<0.01$). Memory perfor-

mance was strongly correlated to hippocampal glutamine ($r=0.62$, $P<0.001$) and taurine ($r=-0.526$; $P<0.001$).

Conclusion: These data demonstrate that insulin-resistant GK rats are characterised by brain structural deterioration and metabolic modifications. Some metabolites of the measured neurochemical profile were associated to memory performance and therefore constitute non-invasive biomarkers of brain dysfunction in diabetic encephalopathy.

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Weight loss following bariatric surgery recovers decreased brain volumes but not increased fatty acid metabolism in the morbidly obese: a combined PET and VBM study

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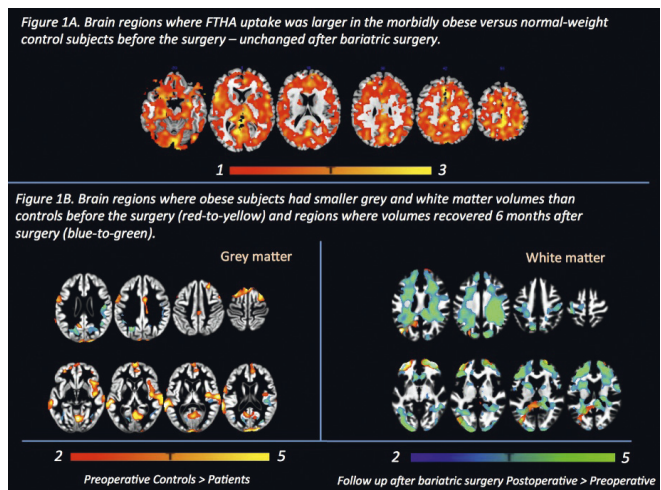
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Background and aims: Obese individuals have widespread cortical and sub-cortical atrophy in white (WM) and grey matter (GM). This may be due to metabolic changes that divert brain energy metabolism to abnormally high utilisation of glucose or fatty acids (FA) leading to increased oxidative stress and cellular damage. We studied the effects of weight loss due to bariatric surgery on brain FA uptake and GM / WM densities in obese subjects with and without type 2 diabetes. We hypothesized to find decrease of FA uptake and increase in WM and GM after the surgery.

Materials and methods: We studied 20 morbidly obese subjects before (meanBMI 41; SD 3,4) and six months after bariatric surgery (meanBMI 31,8; SD 4,2), and 13 age-matched healthy subjects (meanBMI 22,6; SD 2,8) with palmitate analogue [18F]-FTHA positron emission tomography. Brain fatty acid (FA) uptake was calculated using Gjedde-Patlak analysis. For the voxel-based morphometric analysis the T1-weighted MRI images were segmented into WM and GM using the VBM8 toolbox. Group differences in FA, WM and GM images were compared using independent samples t test in SPM8. Voxelwise associations between FA uptake and WM and GM volumes were analysed using Pearson correlations.

Results: Preoperatively, brain FA uptake was higher in morbidly obese versus lean subjects across the whole brain (Figure 1A) and this was not influenced by weight loss following surgery. Preoperatively the morbidly obese subjects had globally lowered grey and white matter density and both recovered following the surgery (Figure 1B). White or grey matter volumes and FA uptake were not associated. The observed differences were independent of diabetic state of the participants.

Conclusion: We show here that fatty acid uptake is increased in morbid obesity and that the increase coincides, but is not associated, with adverse structural changes in white matter. Brain atrophy reversed already within 6 months after bariatric surgery whereas no changes occur in brain FA uptake. This suggests that increased FA fluxes do not serve potential reversible causes of brain morphological changes in obesity and diabetes, but could be due to obesity related increased brain insulin sensitivity, which is normalized after bariatric surgery.



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PS 047 Adipose tissue function in humans

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Obesity decreases the expression of sirtuin proteins in adipose tissue of monozygotic twins, independent of genetic factors

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Background and aims: Sirtuins 1 and 3 (SIRT1, 3) are reported to be down-regulated in the subcutaneous adipose tissue (SAT) of obese individuals. Some studies associate human SAT SIRT1 and 3 expression with a favorable obesity phenotype. How much all of these associations reflect acquired obesity or are confounded by genetics and other factors is unclear. The objective of this study was to assess the roles of SAT SIRT1, 3 and 5 expression in obesity, insulin resistance and inflammation, controlling for genetic and early environmental factors by studying monozygotic (MZ) twins.

Materials and methods: Global gene mRNA expression, body composition and insulin sensitivity were assessed with Affymetrix U133 Plus 2.0 arrays, magnetic resonance imaging (MRI), DEXA scans and 75g oral glucose tolerance tests (OGTT) in healthy MZ twin pairs (N = 40) of which 26 pairs were discordant for BMI (BMI difference > 3 kg/m²). For statistical analyses, Δ -variables depicting intrapair differences in different traits were calculated by subtracting the value of the leaner co-twin from the value of the heavier co-twin. These Δ -variables were used to control for genetic and early environmental factors that the twins share.

Results: SIRT1, SIRT3 and SIRT5 were significantly downregulated in the SAT of heavier co-twins of BMI-discordant pairs. Out of adiposity variables, Δ intra-abdominal fat volume was the best predictor of Δ SIRT1 expression ($r = -0.81$, $P < 0.001$). Where SIRT3 and 5 were correlated with adiposity in the individual level ($r = -0.30$ to -0.55 , $P < 0.007$), these associations disappeared in the intrapair differences control setting ($r = -0.01$ to -0.25 , $P > 0.114$). Δ SIRT1 and 5 expression was associated with insulin sensitivity during OGTT ($r = -0.32$ to -0.47 , $P < 0.047$). Δ SIRT1 and 5 were significantly correlated with Δ CRP levels, Δ CD14 expression and various inflammatory gene expression pathways in SAT ($r = -0.29$ to -0.52 , $P < 0.042$). Contrary to earlier research, Δ SIRT1 mRNA was not correlated with Δ adiponectin mRNA ($r = 0.12$, $P = 0.469$).

Conclusion: Adiposity (especially IA fat) was strongly related to SAT SIRT1 independent of genetic or early environmental influences, whereas SIRT3 and 5's association with adiposity seems to be confounded by genetic or early environmental factors. SAT SIRT1 and 5 are negatively associated with insulin resistance and adipose tissue inflammation, even after controlling for twin-shared factors.

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Obesity is associated with lower expression of mitochondrial respiratory chain and mitoribosomal transcripts in adipose tissue in monozygotic twins

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Background and aims: Low mitochondrial number and activity are suggested to be underlying factors in obesity and metabolic syndrome. However the functions of mitochondrial ribosomes, which are responsible for the synthesis of respiratory chain proteins, in acquired obesity are largely unknown.

Materials and methods: Rare young adult MZ twin pairs discordant ($n = 26$, Δ BMI > 3 kg/m²) and concordant for obesity ($n = 14$, Δ BMI < 3 kg/m²) identified from ten full birth cohorts of 22–32-year-old Finnish twins were examined. Abdominal body fat distribution was measured by magnetic resonance (MR) imaging, liver fat content by MR spectroscopy and insulin sensitivity

by the oral glucose tolerance test (OGTT). Mitochondrial ribosomal protein (MRP) expression (Affymetrix U133 Plus 2.0) and respiratory chain (RC) protein levels (Western) were quantified from subcutaneous adipose tissue biopsies.

Results: The obese (BMI 31.2±1.0 kg/m²) and lean (25.3±0.9 kg/m²) co-twins of the discordant pairs had a mean 18±0.5 kg, $p < 0.001$ difference in the body weight. The obese co-twins had significantly more subcutaneous (3810±420 vs. 6360±540 cm³, $p < 0.001$), intra-abdominal (1640±250 vs. 790±180 cm³, $p < 0.001$) and liver fat (4.5±0.9 vs. 1.1±0.3%, $p < 0.001$), and were more insulin resistant (HOMA-index 2.1±0.3 vs. 1.1±0.1, $p < 0.01$), (AUC insulin 130±25 vs. 88±8, $p = 0.055$), (Matsuda-index 5.9±0.7 vs. 8.6±0.9, $p = 0.02$). They also had significantly lower expression levels of mitochondrial ribosome subunits in the adipose tissue, both small (MRPS mean ± SE -0.31±0.1) and large (MRPL 0.26±0.1) than the lean co-twins (MRPS 0.03±0.09, $p < 0.001$, MRPL = -0.04±0.1, $p = 0.0021$). MRPS expression in individuals correlated negatively with HOMA-index ($r = -0.45$, $p < 0.001$), AUC insulin during OGTT ($r = -0.57$, $p < 0.001$), fasting triglycerides ($r = -0.50$, $p < 0.001$), and positively with Matsuda-index ($r = 0.38$, $p = 0.01$) and adiponectin ($r = 0.53$, $p = 0.02$). Large subunits (MRPL) correlated with AUC insulin ($r = -0.45$, $p < 0.001$), triglycerides ($r = -0.41$, $p < 0.001$) and adiponectin ($r = 0.43$, $p = 0.04$). The levels of RC complexes that are partly encoded by mitochondrial DNA were significantly reduced in the obese co-twins' fat (RC/β-TUBULIN i.e. RC per cellular proteins: CI $p = 0.042$, CIII $p = 0.015$, CIV $p = 0.019$, CV $p = 0.036$) compared to the lean cotwin. The amount of CI ($p = 0.050$), CIII ($p = 0.011$) and CIV ($p = 0.037$) were reduced also per mitochondria (RC/PORIN) in the obese co-twins. However, CII, which is encoded by nuclear DNA, was not different between the discordant co-twins. No differences were observed between the twins from the concordant pairs.

Conclusion: Mitochondrial ribosome protein expression and respiratory chain protein levels in adipose tissue are decreased in acquired obesity, together with a reduction in whole body insulin sensitivity. This suggests that down-regulation of mitochondrial biogenesis in adipose tissue plays a major role in the development of metabolic complications in obesity.

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Knock down of hormone-sensitive lipase in human adipocytes improves glucose metabolism via induction of de novo lipogenesis

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Background and aims: We recently showed that partial inhibition of adipose tissue lipolysis improves glucose metabolism and insulin sensitivity in vivo. Adipose tissue de novo lipogenesis (DNL) has recently emerged as a determinant element of whole body insulin sensitivity. DNL enzymes are under the control of the transcription factor carbohydrate-responsive element binding protein (ChREBP). Here, we aimed at understanding the specific role of de novo lipogenesis (DNL) in the improvement of human adipocyte glucose metabolism when hormone-sensitive lipase (HSL) expression is diminished.

Materials and methods: Human mesenchymal adipose-derived stem (hMADS) cells were differentiated into adipocytes during 14 days of culture, and then used for metabolic analysis. Knock down was achieved by microporation of small interfering RNA against HSL (siHSL), ChREBP (siChREBP), and as a control green fluorescent protein (siGFP). Gene expression was quantified by RT-qPCR. Radiolabelled 2-deoxy-glucose was used to measure glucose uptake. Glucose oxidation and DNL (glucose carbon incorporation into fatty acids) were evaluated using radiolabelled glucose. Analyses of fatty acid composition in triglycerides and phospholipids were performed using capillary gas chromatography. Cells membranes were separated in 12 different fractions by sucrose gradient, and lipid raft composition was analyzed by Western blot. GM1 ganglioside was detected by Cholera toxin B.

Results: Compared to control, glucose transport and oxidation were increased in siHSL adipocytes. This improvement of glucose metabolism was accompanied by an increase of DNL both at the levels of key enzymes gene expression and metabolic flux. Notably, there was a marked induction of ChREBP in siHSL adipocytes. To establish the role of DNL in the improved glucose re-

sponse, ChREBP expression was knocked down. Although siChREBP alone did not affect glucose metabolism, we observed a significant decreased glucose transport, glucose oxidation and DNL in cells exposed to dual inhibition (siHSL/ChREBP) compared to simple inhibition (siHSL), suggesting an involvement of ChREBP in the effects mediated by HSL down regulation. Cellular lipidomic analyses demonstrated a clear qualitative change in fatty acid composition of phospholipids (PL) and triglycerides (TG) between the different siRNA conditions. The knock down of HSL led to an increase proportion of oleate (in PL and TG), in parallel to a decreased proportion of palmitoleate, while the knock down of ChREBP had the opposite. An intermediate profile was observed with dual inhibition (siHSL/ChREBP). These results could partly be explained by the changes in the expression of ELOVL6, an elongase responsible for the shift between C:16 to C:18 series. Interestingly, these changes in PL composition could contribute to the observed changes in lipid rafts associated to insulin signaling.

Conclusion: These data concur to a prominent role of DNL in the improved glucose metabolism observed when adipocyte HSL expression is diminished. Supported by: ANR ObeLip

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High fat diet induced adipose tissue inflammation by suppression of anti-inflammatory phospholipids

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Background and aims: Little is known about the interdependency of complex gene-networks regulating the metabolism or signaling function of specific lipids in humans. Lipid metabolism and signaling might be either controlled genetically or by environmental factors. The aim of the study was to correlate lipidomic and genomic data of human subjects to identify specific gene-modules responsible for the regulation or connected with the function of specific lipid metabolites.

Materials and methods: In the NUGAT-Study (NUtriGenomic Analysis in Twins) all subjects (monozygous and dizygous twins) first received a carbohydrate-rich low-fat diet for 6 weeks (Clinical Investigation Day 1, CID1 thereafter), immediately followed by a high-fat diet for 1 week (CID2) and additional 5 weeks (CID3). At each CID periumbilical fat biopsies were taken for RNA isolation and Agilent 8x40K gene micro Arrays. Plasma was measured for lipid metabolites and cytokines (ELISA). Weighted gene Co-Expression Network Analysis (WGCNA) was used for identification of co-expressed gene-networks and their correlation with lipidome data. MetaCore was used to find potential roles of identified gene-sets.

Results: By analysis of the 5000 strongest regulated genes 10 gene-modules were identified whereof 1 revealed a high correlation ($p < 0.00001$ to 0.0006) with CRP ($r^2 = 0.31$), VEGF ($r^2 = 0.39$), IL1ra ($r^2 = 0.35$) and PP ($r^2 = 0.3$). This gene-module contained genes which are known to play a role in processes like phosphatidylethanolamine acyl-chain remodelling, immune response, response to wounding, platelet activation and cytokine production. Furthermore, the gene-module was highly associated ($r^2 = -0.32$, $p < 0.0004$) with a lysophosphatidylethanolamine-metabolite (LPE), a phospholipid which has been previously suspected for its anti-inflammatory/anti-bacterial functions. In almost all volunteers LPE decreased during the high-fat diet (mean: $-0.50/-0.70$ mmol/L), as well as IL1ra, while CRP and VEGF increased. Interestingly, the concentration of VEGF was very similar in monozygous twins, the heritability was calculated to approx. 90% during all CIDs.

Conclusion: While LPE and IL1ra, which is an anti-inflammatory cytokine, decrease during the high-fat diet, CRP and VEGF, which are markers for inflammation and migration of monocytes/macrophages, increase. Our data show for the first time in humans that high-fat diet induces inflammation by remodeling lipid biosynthetic pathways reducing anti-inflammatory and increasing inflammatory signaling. Specifically we identify downregulation of the anti-inflammatory phospholipid LPE as a mediator of inflammatory processes in the adipose tissue.

Clinical Trial Registration Number: NCT01631123

Supported by: BMBF, DZD

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Adipose tissue as an extrathyroidal source of TSHbeta

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Background and aims: In addition to the pituitary-thyroid circuit, additional TSH-related circuits may be functioning in extrathyroidal sites, including the immune system as evidenced by the ability of immune cells to produce TSH. A splice variant of TSH β has been identified in the pituitary gland, thyroid and peripheral blood leukocytes.

Materials and methods: Either total (t-) or variant (v-) TSH β gene expression were studied in 40 visceral (VAT) and in 42 subcutaneous adipose tissue (SAT) samples. The expression pattern was also evaluated in adipose tissue fraction cells (mature adipocytes (MA) and stromal vascular fraction (SVF)) and during adipocyte differentiation.

Results: Either t- or v-TSH β were consistently detected in adipose tissue. t- and v-TSH β expression were similar in MAs and SVFs. In SAT, t-TSH β decreased during adipocyte differentiation. Perilipin 1, perilipin 2 (ADRP), FSP27 and several lipolytic gene expression (ATGL and MGLL) were negatively associated with t- and v-TSH β in both SAT and VAT. Conversely, t- and v-TSH β were positively associated with caveolin1 in SAT and VAT. Moreover t- and v-TSH β from AT were positively associated with PGC1a and SIRT1 gene expression. Both TSH β isoforms in AT were associated with an adverse lipid profile.

Conclusion: Current results provide novel evidence of adipose tissue as an extrathyroidal source of TSH β in association with genes implicated in lipid droplet formation and lipolysis.

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Coxsackie and adenovirus receptor: novel depot-specific molecule in human adipose tissue?

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Background and aims: The Coxsackie and Adenovirus receptor (CAR) plays a crucial role in both virus-related pathology and adenoviral gene therapy. Recent findings disclose important functional roles in immunity, inflammation, and tissue homeostasis.

Materials and methods: CAR gene expression was measured in two-hundred human adipose tissue samples (91 visceral (VAT) and 109 subcutaneous adipose tissue (SAT)). CAR was also studied in human adipose tissue fractions (mature adipocytes (MAs) and stromal vascular fraction (SVF)).

Results: CAR expression was 60-fold higher in VAT than in SAT (0.036 ± 0.021 vs 0.0006 ± 0.0005 , $p < 0.0001$). In human adipose tissue fractions CAR gene expression in SVF was significantly higher than in MAs, either in SAT (0.0016 ± 0.0002 vs 0.00038 ± 0.00004 ; $p = 0.005$) or in VAT (0.0654 ± 0.0043 vs 0.0249 ± 0.0040 , $p = 0.028$). In SAT, CAR expression was higher in obese than in lean subjects (0.00068 ± 0.00060 vs 0.00048 ± 0.00036 , $p = 0.019$), whereas in VAT this pattern was only observed in men (0.043 ± 0.022 vs 0.017 ± 0.022 , $p = 0.031$). In SAT, CAR was positively associated with BMI ($r = 0.258$; $p = 0.008$), fat mass ($r = 0.288$; $p = 0.003$), and inversely with the expression of genes such as PPAR γ ($r = -0.381$; $p = 0.035$), FAS ($r = -0.333$; $p = 0.011$) and Srebp-1c ($r = -0.357$; $p = 0.019$). In VAT, CAR was also positively associated with BMI ($r = 0.460$; $p = 0.036$) only in men, whereas negatively with the expression of the lipogenic gene S14 ($r = -0.260$; $p = 0.019$) and GLUT4 ($r = -0.531$; $p = 0.001$) and FABP5 ($r = -0.350$; $p = 0.015$).

Conclusion: The Coxsackie and Adenovirus receptor is detected in both adipose tissue depots, being preferentially expressed by VAT and stromal vascular cells. CAR expression was increased in AT from obese subjects, mainly in SAT, in inverse association with different lipogenic genes.

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Human adipose tissue microvascular endothelial cells (MVEC) regulate both tissue lipid in-/efflux and adipose cell PPAR γ activation

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Background and aims: Animal studies have shown that specific deletion of PPAR γ in endothelial cells (EC) leads to dyslipidemia and endothelial dysfunction. We have previously established a method to isolate and culture human primary MVEC from adipose tissue biopsies. We here asked if the MVEC and (pre)adipose cells cross-talk in the regulation of the bidirectional transport of fatty acids between the blood and the adipose cells. We also examined the putative cross-talk between MVEC and PPAR γ activation in the adipocytes which, in turn, is required to store excess fat in the adipose cells.

Materials and methods: MVEC were extracted from human subcutaneous adipose tissue biopsies using immunomagnetic separation with anti-CD31 Dynabeads. Selected MVEC were cultured and expanded in EC growth medium and the remaining preadipocytes in DMEM-F12 prior to differentiation. Subsequently, these cells were co cultured. To promote lipid accumulation, the MVEC were incubated with 300 μ M oleic acid (OA) and/or 5 μ M Rosiglitazone (ROSI) for 48 h. Accumulated lipids in the cells were visualized with the fluorescent long-chain fatty acid analogue BODIPY-500/506 C1 C12 and microphotographs were taken with fluorescence or confocal microscopes. PPAR γ activation was identified with the cell-based PPAR γ reporter assay-GeneBLazer UAS-bla HEK 293Hcells.

Results: Both lipid transporters CD36 and FABP4 were highly expressed in MVEC and they were further markedly increased by the addition of the PPAR γ agonist ROSI. Interestingly, a similar effect was seen by just adding oleic acid (OA) which in turn, also increased PPAR γ gene activation in MVEC and there was no additive effect of combining OA with ROSI. Furthermore, the stimulatory effect of OA was significantly inhibited by the PPAR γ inhibitor GW9662, indicating that OA directly activated PPAR γ in MVEC. We also examined the cross-talk between MVEC and adipose cells in co-cultures and confirmed the presence of a regulated bidirectional lipid transport (flux and efflux) between these cells. We then investigated the effect of OA in cultured adipocytes and, in contrast to MVEC. OA did not directly increase CD36, FABP4 or PPAR γ expression. However, in co-cultures, the addition of the MVEC insert increased the expression of both lipid transporters as well as that of PPAR γ suggesting that MVEC secreted a PPAR γ agonist to which the adipocytes responded. To further verify this, we incubated the PPAR γ reporter cells (reporter assay) with MVEC conditioned media or with equivalent naïve media. Indeed, PPAR γ activation was significantly increased when the cells were incubated with MVEC conditioned media as compared with naïve media. This was consistent for both basal culture medium and was further increased by the OA-containing media.

Conclusion: These findings support the presence of an intimate cross-talk between adipose cells and MVEC in the regulation of the bidirectional transport of fatty acids. Furthermore, we can here also show for the first time that MVEC secrete a PPAR γ ligand targeting both the MVEC and the adipose cells and that this process is further increased in the MVEC by fatty acids.

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The effect of 2 days very-low calorie diet on cytokines in adipose tissue and in plasma in obese women

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Background and aims: A number of bioactive molecules produced by adipose tissue (AT), such as cytokines, chemokines and acute phase proteins, have been suggested as a possible link between obesity and insulin resistance. It has been shown that circulating adipokines are elevated in obese and diabetic subjects. Very-low-calorie diets (VLCD) are used as dietary intervention to achieve a rapid weight loss in obesity treatment and were shown to improve insulin sensitivity (IS) as soon as after 2 days of intervention. The aim of our study was to investigate the response of pro- and anti-inflammatory cytokines in AT and plasma after 2 and 28 days of VLCD and its possible relationship to IS improvement.

Materials and methods: 16 obese pre-menopausal women (BMI 32.5 ± 3.6 kg/m²) followed 800 kcal/d VLCD for 28 days. Anthropometric measurements, blood sampling and biopsy of subcutaneous abdominal AT were performed before diet and at the day 2 and 28 of VLCD. To evaluate insulin sensitivity, euglycemic-hyperinsulinemic clamp was performed at each phase and Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated. IL-6, IL-1 β , IL-8, IL-10, MCP-1, and TNF α , were analyzed in plasma and in conditioned media of AT explants using multiplex immunoassay at Luminex 100. AT mRNA expressions of cytokines and lipogenic genes DGAT2, SDC, and FASN were analyzed.

Results: At day 2 IS increased as evaluated by HOMA-IR but not by glucose disposal rate. At day 28 both indices of IS increased. Fat mass was not changed at day 2, while it decreased at day 28 (38 ± 8.5 kg vs. 32 ± 7 kg). At day 2, levels of IL-1 β , IL-6 and IL-10 were increased in plasma, while the secretion and mRNA expression of all cytokines in AT was unchanged. In contrast, at day 28, secretion and mRNA expression of all cytokines in AT was increased, while their plasma levels were not different from the pre-diet condition. Expression of lipogenic genes DGAT2, SCD and FASN decreased at day 2 (DGAT2, $p=0.04$; SCD, $p=0.005$; FASN, $p=0.05$) and their decrease was further enhanced at day 28 ($p=0.001$ for all genes).

Conclusion: Improvement of IS after 2 days of VLCD was not associated with changes in expression and secretion of cytokines in AT. The concomitant increase in plasma cytokines suggests a contribution of non-adipose tissues. The lipogenesis in AT was down-regulated at the early phase of the diet.

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The number of microRNA target sites identifies those transcripts that will experience the most dramatic downregulation in adipose tissue from obese subjects after weight lossF.J. Ortega¹, J.M. Mercader², J.M. Moreno-Navarrete¹, L. Nonell³, E. Puigdecant³, G. Xifra¹, M. Sabater¹, M. Moreno¹, N. Fuentes-Batllevell¹, D. Mayas⁴, N. Moreno-Castellanos⁵, W. Ricart¹, F.J. Tinahones⁴, M. Malagón⁵, J.M. Fernández-Real¹;¹Department of Diabetes, Endocrinology and Nutrition (UDEN), Institut d'Investigació Biomèdica de Girona (IdIBGi), ²Joint IRB-BSC program on Computational Biology, Barcelona, ³Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, ⁴Service of Endocrinology and Nutrition, Hospital Clínico Universitario Virgen de Victoria de Malaga, ⁵Department of Cell Biology, Physiology and Immunology, Instituto Maimonides de Investigaciones Biomedicas de Cordoba (IMIBIC)/Reina Sofia University Hospital, University of Cordoba, Spain.

Background and aims: Bariatric surgery is an excellent therapeutic approach to elucidate the pathophysiology of obesity-associated metabolic disturbances. The identification of abnormal function and gene expression patterns regulated by multiple microRNAs (miRNAs) may be useful in this context. In this study we aimed to identify changes in the genome wide adipose tissue (AT) transcriptome linked to the miRNA profile after surgery-induced weight loss.

Materials and methods: Whole genome and miRNA expression patterns were assessed in subcutaneous AT of 16 morbidly obese women before and after surgery-induced weight loss. Validation of both genome wide and miRNA microarrays was made by quantitative real-time PCR using both longitudinal and cross-sectional cohorts. Three alternative datasets were downloaded from public repositories and analyzed to check for reproducibility. Analyses in macrophages and differentiated human adipocytes were performed in vitro to try to comprehend the associations found in human AT.

Results: Five thousand and eighteen different probe sets identified significant variations in gene expression. Only 15 miRNAs differed in the comparison before-after surgery-induced weight loss. Functional analysis revealed significant changes in cell cycle, development and proliferation, in lipid metabolism, and the inflammatory response, as further confirmed by in vitro results. Interestingly, when transcriptomes were analyzed taking into account the presence of miRNA target sites, the mRNAs that experienced the most dramatic changes were precisely those with more miRNA target sites ($p=10^{-195}$), being significantly down-regulated after surgery-induced weight loss.

Conclusion: Current findings suggest that surgery-induced weight loss is associated with an improvement in post-transcriptional modulation by miRNAs, in parallel to changes in inflammatory status and lipid metabolism of obese AT.

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Relationship between muscle/adipose tissue morphology, insulin sensitivity and beta cell function in diabetic and nondiabetic obese patients: effects of bariatric surgery

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Background and aims: Obesity is associated with low-grade inflammation in adipose tissue (AT), high lipolytic activity, ectopic fat deposition and insulin-resistance (IR). Bariatric surgery leads to major fat mass loss and improvements in IR and β -cell function (β -GS). Aim of this study was to relate the improved metabolic status after Roux-en-Y gastric bypass (RYGB) to the morphological changes of subcutaneous (SAT) and visceral AT (VAT) and skeletal muscle.

Materials and methods: 14 non-diabetic (ND) and 14 type 2 diabetic (T2D) obese patients (BMI = 50 ± 2 and 52 ± 2 kg/m²) received a euglycaemic clamp study (to measure IR) with 2H5-glycerol infusion (to measure lipolysis) and a mixed meal test (to measure insulin secretion and β -GS) before and one year after RYGB. During RYGB, VAT (omental), SAT and rectus abdominis samples were excised for light (LM) and electron microscopy (EM) analysis.

Results: Before surgery, both T2D and ND patients showed marked IR and enhanced lipolysis, T2D also had impaired β -GS. On LM, perivascular and interfibrillar muscle fat content was similar in ND and T2D; intramyocellular fat was more abundant in T2D than ND patients (1.0 [1.5] vs 0.1 [0.5] units, $p=0.008$). In SAT, adipocyte area and density of crown-like structures (CLS) were similarly increased in T2D and ND. In VAT, adipocyte area (5806 [1793] vs 5056 [1320], $p=0.01$) and CLS density (3.60 [7.31] vs 0.00 [1.75], $p=0.0002$) were higher in T2D than ND. β -GS was inversely related to VAT adipocyte area ($r = -0.58$, $p=0.004$) and CLS density ($r = -0.50$, $p=0.02$). On EM, SAT and VAT adipocytes showed necrotic material, fibrosis, thickened capillary basal membrane, degenerating adipocytes with thin cytoplasm with extrusion of free lipids in interstitium and small mitochondria. In T2D patients, VAT and SAT blood capillaries contained neutrophils. After RYGB (33% weight loss), IR and lipolysis were markedly improved, equally in ND and T2D ($p<0.003$ for all). In T2D, β -GS was improved ($p=0.04$) but not normalized. By LM, in both ND and T2D fat deposits were reduced in all muscle locations ($p<0.03$) as were adipocyte area and CLS density in SAT ($p<0.0001$). By EM, SAT adipocytes were generally smaller, in advanced state of delipidation, with thicker cytoplasm rim and more mitochondria. In T2D, capillaries were free of neutrophils. In the whole dataset, SAT adipocyte area and CLS density were strongly related to BMI, IR and lipolysis (r 's between 0.40 and 0.81, all $p<0.0001$). β -GS was related to SAT CLS density ($r = 0.46$, $p=0.006$) and intramyocellular fat ($r = 0.53$, $p=0.001$); the post-surgery increase in β -GS was related to the concomitant decrease in intramyocellular fat ($r = -0.79$, $p=0.002$).

Conclusion: In morbid obesity, fat accumulation results in adipocyte enlargement, lipid extrusion, macrophage infiltration and cell necrosis. In both T2D and ND, these changes correlate with IR and lipolysis; in VAT, histology is worse in T2D than ND and correlates with β -cell dysfunction. After surgically-induced major weight loss, AT/muscle histology and IR improve in parallel; in T2D, however, β -cell glucose sensitivity remains abnormal despite the restoration of tissue morphology, suggesting a different origin for β -cell incompetence.

PS 048 Brown, beige or brite?

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The lipid pattern of brown adipose tissue is distinct from white fat and shows sex-specific differences in mice

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Background and aims: Brown adipose tissue (BAT) has a vital function as organ of non-shivering thermogenesis in small mammals and neonates and may also be of relevance in adult humans. Besides the unique expression of uncoupling protein 1, BAT also differs from white adipose tissue (WAT) by containing more mitochondria and multilocular lipid droplets. To obtain a more detailed understanding of the differences between WAT and BAT on the level of lipid metabolism, we performed an extensive lipid profiling of BAT and two different WAT depots, gonadal (GAT) and subcutaneous adipose tissue (SAT).

Materials and methods: Interscapular BAT, femorogluteal SAT and GAT were quickly dissected from 12 C57Bl/6N mice (6 female and 6 male, 11 week-old) following anaesthesia and decapitation. For lipid extraction, 10 mg of frozen fat were homogenized in 75% ethanol and further extracted with MTBE. Nontargeted lipidomics analysis was performed on a Waters ACQUITY UPLC system coupled with an ABI Sciex tripleTOFTM 5600 plus mass spectrometer. High resolution tandem MS was utilized to enhance lipid identification. All detected lipids were quantified by normalization to internal standards.

Results: In total, 329 lipid species from 15 classes, covering FFA, DG, TAG and several phospho- and sphingolipid classes, could quantitatively be detected. In addition, acyl (alkenyl, alkyl) chains could be assigned to all phospho- and sphingolipids. Overall, WAT contained significantly more TAG while BAT contained more phospholipids and sphingomyelin. Interestingly, 6 of the 11 lipids (9 PE- and 2 PC-species) that showed the highest specificity for BAT contained docosahexaenoic acid. The key differences between the two WAT depots were a higher amount of TAG and lower content of several phospholipid classes in GAT. Interestingly, multivariate PCA analysis led to a clear separation of BAT samples from female and male mice. By analysing the acyl chain composition of the lipids contributing most to this separation, a set of fatty acids including arachidonic and stearic acid was found to occur more frequently in females, while another set including linoleic and palmitic acid was higher in males. Despite the differences between female and male GAT that could be expected in the light of their different anatomical location (ovarial/epididymal), the lipid composition of GAT was less sex-specific than the one of BAT.

Conclusion: By MTBE-based extraction followed by high-resolution LC-tandem-MS analysis, we were able to generate detailed lipid patterns of brown and two white adipose tissue depots of female and male mice. When comparing the lipid composition of the different depots, the key findings were, firstly, clear adipose type-specific differences between BAT and WAT, and, secondly, sex-specific differences in phospholipid composition which were most prominent for BAT. By obtaining the composition of all phospho- and sphingolipids, we could identify common FFA residues characterizing the most discriminant lipid species, a prerequisite to explain the metabolic processes underlying the depot- and sex-specific differences observed.

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Peri-droplet mitochondria in brown adipocytes form an exclusive subpopulation of mitochondria

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Background and aims: Mitochondria play essential roles in brown adipose tissue differentiation and function. We have recently shown that mitochondrial dynamics is a physiological regulator of adrenergically-induced changes in energy expenditure. Moreover, we and others report that changes in mi-

tochondrial architecture may represent adaptive changes to bioenergetics needs. Conditions requiring high mitochondrial ATP synthesis capacity and/or efficiency are associated with mitochondrial elongation. Whereas conditions of excess energy supply and low ATP demand acutely induce mitochondrial fragmentation. However it remains unclear if diversity in subcellular mitochondrial architecture may play a role in generating functional specialization of subpopulations of mitochondria within the cell. We rationalized that the brown adipocyte may shed light on this question as its mitochondria are required for various and competing tasks, such as lipogenesis and beta oxidation as well as ATP synthesis and uncoupling, raising the possibility that the brown adipocyte may harbor a diverse set of mitochondria which are structured to fit different functions.

Materials and methods: Brown pre-adipocytes were harvested from 3-week-old male C57BL/6J mice and differentiated in culture. Mitochondrial membrane potential, motility and morphology were measured using TMRE and PAGFP respectively with Zeiss LSM 710 confocal microscope. NADH autofluorescent was used to measure the NADH content of the mitochondria. Protein import and turnover were measured using MitoTimer probe.

Results: Mitochondria in brown adipocyte are divided to two different populations, mitochondria with submicron proximity to the lipid droplet or Peridroplet (PD) mitochondria, and mitochondria that are located at least 5 µm away from the vicinity of a lipid droplet or Cytoplasmic (C)mitochondria. Photo-conversion of matrix targeted photoactivatable GFP shows that these two populations rarely mix and that mitochondria remain faithful to the lipid droplet they adhere to and do not share their matrix content with other mitochondria, nor do they switch their affiliation from one group to another. These studies also show that PD-mitochondria are more elongated while the C-mitochondria tend to be smaller in size suggesting a more coupled respiratory function and involvement in ATP synthesis. In addition, using TMRE, we found that PD-mitochondria in brown adipocytes but not in other lipid-containing cells are in different energetic state than C-mitochondria. Ratio-metric imaging of membrane potential and NADH/NAD ratio show that PD-Mitochondria have higher NADH/NAD ratio and more polarized membrane potential. Protein import and turnover studies using MitoTimer also indicate that PD-mitochondria have a higher rate of protein import and biogenesis, in agreement with the hyperpolarized state. Altogether these observations support a hypothesis that PD-mitochondria have a higher TCA cycle activity.

Conclusion: These data suggests that peridroplet mitochondria represent a functionally exclusive subpopulation of mitochondria in terms of biogenesis and function. This may also demonstrate that the unique architectural characteristics of cells in diverse bioenergetics states may apply subcellularly to a diverse set of mitochondria within the cell, fulfilling different metabolic functions.

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Diet-induced interleukin-15 promotes obesity by inhibiting adaptive thermogenesis in adipose tissues

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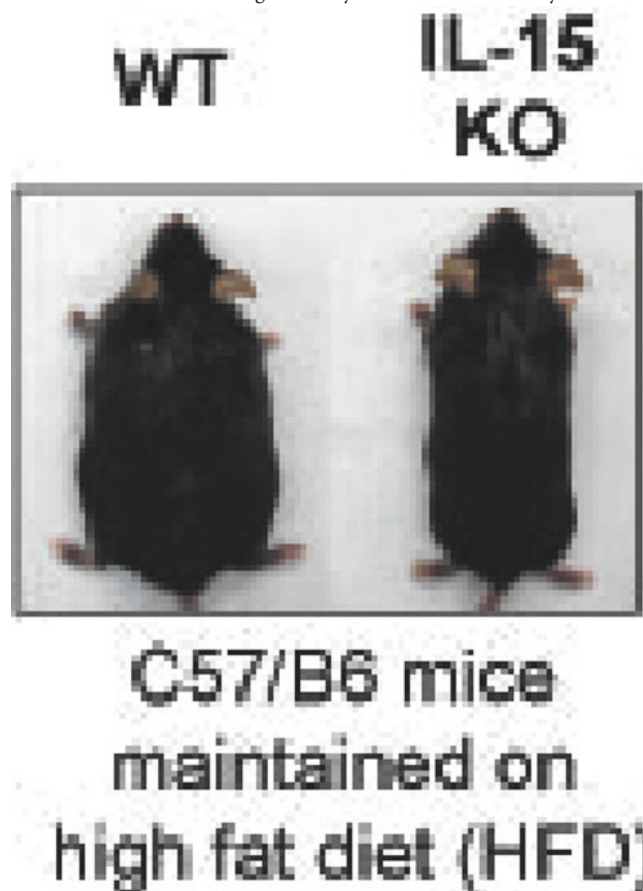
Background and aims: Inflammatory cytokines are implicated in the pathogenesis of obesity. Interleukin-15 (IL-15) is an inflammatory cytokine secreted by many cell types. IL-15 is also produced during physical exercise by skeletal muscle and has been reported to reduce weight gain in mice. Contrarily, our findings on IL-15 knockout (KO) mice indicate that IL-15 promotes obesity. The aim of this study is to investigate the molecular and cellular mechanisms underlying the pro-obesity role of IL-15 in adipose tissues.

Materials and methods: C57BL/6 wildtype and IL-15 KO mice were maintained on high fat diet (HFD) or normal control diet. After 16 weeks, body weight, adipose tissue and skeletal mass, serum lipid levels and gene expression in the adipose tissues were evaluated. Primary cultures of brown and white adipocytes were studied using the Seahorse cell metabolism analyzer to evaluate oxygen consumption rates. IL15 gene expression and the expression of inflammation markers were studied in visceral adipose tissues obtained from patients undergoing bariatric surgery.

Results: We show that IL-15 deficiency prevents diet-induced weight gain and accumulation of lipids in visceral and subcutaneous white adipose tissues. Circulating levels of cholesterol and non-esterified fatty acids were elevated in wildtype mice but not in IL-15 KO mice. The adipose tissues of IL-15 KO mice showed decreased expression of pro-inflammatory cytokines, chemokines and macrophage markers CD68 and F4/80. Gene expression

analysis also revealed elevated expression of genes associated with adaptive thermogenesis in the brown and subcutaneous adipose tissues of IL-15 KO mice. Accordingly, oxygen consumption was increased in the brown adipocytes from IL-15 KO mice. Adipose tissues from obese diabetic patients showed a tendency towards increase in the expression of IL-15.

Conclusion: Our results clearly show that IL-15 plays a pathogenic role in obesity. High fat diet-induced IL-15 promotes accumulation of fat in the white adipose tissues by inhibiting lipid utilization via adaptive thermogenesis. IL-15 also promotes inflammation in adipose tissues that could sustain chronic inflammation leading to obesity-associated metabolic syndrome.



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Effect of the n-3 LC-PUFA EPA on white-to-brown transition of primary human adipose-derived stem cells

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Background and aims: Promoting the induction of brite adipocytes and thereby increasing energy expenditure may protect against the development of obesity. We have recently shown that both bone morphogenetic protein (BMP)4 and BMP7 induce browning of primary human adipose-derived stem cells (hASCs). Long-chain polyunsaturated fatty acids (LC-PUFAs) and their metabolites are potential PPARγ ligands and chronic activation of PPARγ is known to promote browning of white adipocytes. Furthermore, dietary n-3 LC-PUFAs have been shown to increase oxidative metabolism in white adipose tissue (AT) as well as adaptive thermogenesis in brown AT, suggesting a possible role for n-3 LC-PUFAs in the white-to-brown transition. Therefore, we investigated the direct effects of the n-3 LC-PUFA eicosapentaenoic acid (EPA) on browning of hASCs in comparison to oleic acid (OA) as non-essential fatty acid and BMP4.

Materials and methods: Primary hASCs were isolated from the subcutaneous depot of different donors and challenged with EPA (20 µM) or OA (20 µM) during adipocyte differentiation (12 days). Cells treated with the known inducer of browning BMP4 (50 ng/ml) served as a positive control.

After 12 days of differentiation, lipid accumulation was measured by Oil Red O staining and gene expression was assessed by qRT-PCR.

Results: Chronic treatment of hASCs with EPA but not OA increased lipid accumulation to similar extents as BMP4. The BMP4-mediated enhancement of PPAR γ and C/EBP α expression was absent in OA-treated hASCs, while the n-3 PUFA EPA slightly increased the expression of PPAR γ and C/EBP α . Neither EPA nor OA affected the expression of the white-specific marker Tcf21, which was significantly reduced by BMP4. Remarkably, UCP1 expression was strongly enhanced (7-fold) after EPA exposure, while OA did not induce UCP1 expression. This EPA-mediated effect on UCP1 expression was comparable to that of BMP4.

Conclusion: In conclusion, chronic exposure to physiological concentrations of the n-3 LC-PUFA EPA induces browning of hASCs derived from white AT, providing a potential mechanism for the beneficial effects of dietary n-3 LC-PUFAs on metabolism. The non-essential fatty acid OA has no effect on white-to-brown transition. The underlying mechanisms of the effect of the essential n-3 fatty acid EPA on browning will be investigated in future studies. *Supported by: Mead Johnson Nutrition, FP7-EU Marie Curie-IEF (ADDIO-PIEF-2012-328793)*

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PPAR γ and PPAR α agonists induce white-to-brown conversion of human white adipocytes along with a metabolic shift from glucose to fatty acid oxidation

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Background and aims: Since it was demonstrated that adult humans possess active thermogenically-competent brown adipose tissue, fighting obesity and its complications through increasing adipose tissue expenditure has emerged as a putative strategy. “Browning” of white adipose tissue in rodents is suggested to be protective against obesity-induced insulin resistance. However, the capacity of human white adipocytes to acquire a brown/beige/brite metabolism has not yet been demonstrated. We aimed at identifying the molecular and metabolic changes associated with the white-to-brown conversion of human mesenchymal adipose-derived stem (hMADS) cells following treatment by PPAR γ (rosiglitazone, Rosi) or PPAR α (GW7647, GW) agonists.

Materials and methods: hMADS cells were differentiated into white adipocytes within 14 days. Thereafter, Rosi or GW were added for 4 additional days before molecular (microarrays, qPCR and Western blot) and metabolic measurements using radiolabelled tracers were carried out.

Results: Both PPAR γ and PPAR α agonists promoted browning of white hMADS adipocytes, as evidenced by higher expression of UCP1 mRNA (29-fold and 18-fold respectively, $p < 0.01$) and protein (18-fold and 13-fold respectively, $p < 0.05$), and of other brown or beige/brite adipocyte markers (CIDEA, PGC-1 α , TBX1, $p < 0.05$). Microarray analysis confirmed by qPCR experiments revealed that treatments stimulated both the fatty acid synthesis and oxidation pathways. Functional measurements were consistent with gene expression data, showing that Rosi and GW increased both fatty acid complete oxidation (by 70% and 66% respectively, $p < 0.05$) and fatty acid incorporation into triglycerides (by 30%, $p < 0.01$ and $p < 0.05$ respectively). The higher fatty acid metabolism was associated with a decrease of both glucose uptake ($p < 0.05$ and $p < 0.01$ with Rosi and GW, respectively) and oxidation ($p < 0.05$ with Rosi and GW). Those metabolic changes occurred without modification of triglyceride cellular content.

Conclusion: PPAR γ and PPAR α activation in differentiated human white adipocytes promotes browning associated with a metabolic shift from glucose to fatty acid oxidation. This striking metabolic remodeling favoring fatty acid use relies on molecular mechanisms which require further investigation. *Supported by: ANR miRBAT, EU FP7 DIABAT*

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EHMT1 and the stimulation with elevated catecholamines can reinduce the perirenal brown adipocytes possessing classical features in adult humans

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Background and aims: Obesity, cause of various metabolic diseases such as type 2 diabetes, results from a chronic imbalance between energy intake and expenditure. Brown adipose tissue (BAT) generates heat against cold and obesity. According to previous reports, the adrenergic stimulation activates the thermogenic program of brown adipocytes. In addition, we previously demonstrated that EHMT1 is an essential methyltransferase in the PRDM16 complex and controls brown adipose cell fate. However, there is a current lack of studies in humans auguring the perirenal BAT and EHMT1.

Materials and methods: We collected adipose tissues from perirenal regions in adult patients with pheochromocytoma ($n = 11$) and those with non-functioning adrenal tumor ($n = 7$). We performed gene expression analysis of perirenal BAT using quantitative RT-PCR and protein analysis by using western blotting for PRDM16, EHMT1 and UCP1. Morphological analyses were performed by H&E staining and immunohistochemistry.

Results: The expression of BAT associated markers including Ucp1 ($p < 0.029$), Cidea ($p < 0.0046$), Elovl3 ($p < 0.015$) were significantly higher in patients with pheochromocytoma. Morphological analysis showed that brown adipocytes were more apparent in patients with pheochromocytoma. These adipose cells possessed the molecular signatures of murine classical brown adipocytes. Furthermore, the upregulation of not only PRDM16 but also EHMT1 accorded with the upregulation of BAT associated markers at both the mRNA and the protein levels.

Conclusion: We find that the stimulation with elevated catecholamines and both PRDM16 and EHMT1 can collaboratively reinduce the perirenal brown adipocytes in adult humans, and these cells have the molecular signatures of classical brown adipocytes.

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MSCA1 is a functional markers of human brite adipogenesis: deleterious effect of inflammation on brite adipogenesis

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Background and aims: Obesity in humans is associated with the accumulation of immune cells in adipose tissue (AT), contributing to systemic low-grade inflammation. The promotion of brite adipogenesis has been shown to ameliorate metabolic parameters in rodents. However, their origin remains to be characterized in humans. Since native CD45-/CD34+/CD31- cells have been previously described as human adipocyte progenitors, the present study aimed to identify the brite-competent progenitor cell subset in humans and to characterize a potential relationship between brite adipogenesis and obesity-associated inflammation.

Materials and methods: Subcutaneous AT were collected from patient undergoing aesthetic surgery for fat removal (cohort1, $n=55$, BMI range 18.4 to 45 Kg/m²). Subcutaneous and matched visceral AT were collected from obese women undergoing gastric bypass (cohort 2, $n=65$, BMI range 33.4 to 62.8 Kg/m²). AT collection was approved by the local ethics committee and donors gave their informed consent. Using flow cytometry approaches with two additional cell surface markers, ie MSCA1, the tissue non-specific alkaline phosphatase, and CD271, a nerve growth factor receptor, we partitioned the AT CD45-/CD34+/CD31- cell population into 3 subsets. We established culture conditions to promote brite adipogenesis using short-term treatment with pharmacological inducers. We developed cell sorting approaches to selectively isolate the progenitor cell subsets. Brite adipogenic potential was assessed by lipid accumulation, gene expression analyses and protein expression of UCP1.

Results: We demonstrated in vitro that brite adipogenesis led to an increase of MSCA1 expression (4-fold increase, $p < 0.05$) and MSCA1 alkaline phosphatase activity (4-fold increase, $p < 0.05$) in human cells. Furthermore, MSCA1 alkaline phosphatase activity was closely associated to the expression of UCP1 and the inhibition of MSCA1 using pharmacological or gene silencing approaches reduced brite differentiation. We showed that human native

immunoselected MSCA1+ cells exhibited brite precursor characteristics: high mitochondrial content (2-fold more compared to other progenitor subsets, $p<0.01$), highest expression of the brite-related precursor genes TMEM26, CD137, CIDEA (2-fold, 3.5-fold and 4.5 fold more respectively compared to other progenitor subsets, $p<0.05$) and they were located predominantly in subcutaneous compared to matched visceral AT. Moreover human MSCA1+ cell subsets exhibited brite adipogenic potential in vitro. Finally, using human cohorts, we provided evidences that MSCA1+ progenitor cells accumulated with obesity and insulin resistance in subcutaneous AT. Using in vitro approaches we demonstrated that immune cells in part through inflammatory cytokines impaired brite adipogenesis by modulating MSCA1 activity.

Conclusion: We demonstrated the existence of brite competent progenitor cells positive for MSCA1 in human subcutaneous AT. However, we highlighted an increase of the proportion of MSCA1+ brite precursor cells in the context of obesity and insulin resistance suggesting a blockade of the terminal differentiation. Such a mechanism could be triggered by immune cells accumulation occurring with obesity.

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BMP7 is produced by a specific human adipose tissue progenitor cell subset and promotes human progenitor cell brite adipogenesis

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Background and aims: Brite adipocytes share some common features with white and brown adipocytes. Adipogenesis leads to the formation of new adipocytes from the differentiation of progenitor cells. Human adipose tissue (AT) progenitor cells, characterized as CD45-/CD34+/CD31-, are a heterogeneous population composed of three distinct progenitor cell subsets. CD45-/CD34+/CD31- progenitor cells have white and brown-like adipogenic potential under pharmacological PPAR γ agonist treatment. However in humans, endogenous signals promoting brite adipogenesis are not clearly known. In mice, bone morphogenetic protein 7 (BMP7) induces brown adipogenesis. The present study was undertaken to characterize BMP7 relevance in human AT and its effects on brite adipogenesis of human native AT progenitor cells.

Materials and methods: Human abdominal subcutaneous AT were obtained from healthy women undergoing aesthetic surgery (body mass index (BMI) 18.4 to 39 kg/m²). AT collection was approved by the local ethics committee and donors gave their informed consent. AT cell populations (adipocytes, endothelial cells, immune cells and progenitor cells) and the three progenitor cell subsets were isolated by immunoselection/depletion approaches. BMP7 gene expression was determined by RT-qPCR analyses in the different AT cell populations and BMP7 protein was studied by western blot. BMP receptor gene expressions were performed by RT-qPCR on isolated AT progenitor cells. BMP7 signaling pathway was studied by western blot. AT progenitor cells were treated (or not, control (ctl)) for 48 hours by BMP7 (50ng/ml) then cultured in basal adipogenic media (without PPAR γ agonist) for 9 days. At day 9, adipogenesis was evaluated by lipid accumulation quantification, brite adipocyte-related gene expressions were performed by RT-qPCR and UCP1 protein was detected by immunohistochemistry and western blotting.

Results: BMP7 gene expression was specifically found in a progenitor cell subset compared to adipocytes, endothelial cells and immune cells ($n=6-10$, $p<0.001$). BMP7 gene expression was positively correlated with BMI ($n=24$, $r=0.59$, $p<0.01$) as well as BMP7 pro-peptide ($n=7$). Human AT progenitor cells expressed BMP receptors ALK2, ALK3, BMPR2 and ACVR2, but not ALK6 ($n=12$). BMP7 stimulation induced phosphorylation of SMAD1/5/8 complex and P38MAPK. Early BMP7 treatment on human AT progenitor cells increased lipid accumulation (2 fold increase vs ctl, $n=7$, $p<0.01$), induced the expression of brite adipocyte-related genes ($n=6$, $p<0.05$) as well as UCP1 protein after 9 days (2 fold increase vs ctl, $p<0.05$).

Conclusion: Our study shows that human AT progenitor cells express BMP receptors and are responsive for BMP7 stimulation. Early BMP7 treatment induces brite adipogenesis on human AT progenitor cells. BMP7 is locally produced by a progenitor cell subset and its expression increases with obesity. These results show that human progenitor cells have a brite adipogenic potential and that BMP7 could be an endogenous brite adipogenic inducer. As brite promotion in rodent has been shown to ameliorate metabolic disorders associated with obesity, local BMP7 might be useful to fight against obesity-associated pathologies in human.

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Brown adipose tissue activity and sirtuin1 expressions in human white adipose tissue during weight loss

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Background and aims: Caloric restriction has been suggested to delay aging-associated disease and extend lifespan through sirtuin proteins. Sirtuins are highly enriched in brown adipose tissue (BAT), but little is known about sirtuins in white adipose tissue (WAT), especially during weight loss. We aimed to study the expression levels of sirtuin1 (SIRT1) in WAT in obese adults during a 12-month weight loss intervention and to correlate these changes with changes in BAT activity.

Materials and methods: 19 obese volunteers (7 males, 12 females) with BMI ranges 29.1-39.0, aged 20-48 years participated in a 12-month weight loss program. 19 healthy lean subjects (7 males, 12 females) with BMI ranges 20.9-24.2, aged 23.7-36.2 years were examined as controls for the obese subjects. The control group was examined similarly as the obese group, however without PET scanning and the weight loss intervention. Total RNA was extracted from subcutaneous WAT biopsies, taken at 0, 5 and 12 months. The whole genome-scale expression profiles were analysed using Affymetrix U133 Plus 2.0 chips. Glucose uptake in BAT was measured in obese patients before and after 5 months, using positron emission tomography (PET).

Results: SIRT1 expression levels were significantly higher ($p<0.001$) in the control group than in obese subjects at the baseline. All obese subjects lost weight between baseline and 5 months (mean $11.6 \pm SE 1.3$ kg). After this, 1/3 of the patients continued to lose weight (weight loss at 12 months 17.5 ± 2.6 kg), while 2/3 of them maintained or slightly regained the weight (mean weight loss at 12 months 5.0 ± 1.2 kg). The expression levels of SIRT1 in WAT followed inversely the BMI trend. Between 0 and 5 months, SIRT1 increased 15% ($p=0.0022$). In those with continuous weight loss, SIRT1 continued to increase at 12 months 6% ($p=0.049$), while among those with weight regain, SIRT1 reverted back to baseline levels. Also, in partial correlation analysis adjusted for sex and age, SIRT1 expression levels correlated negatively with body weight, BMI and liver fat ($r=-0.68-0.60$, all $p<0.001$), and positively with BAT mass ($r=0.53$, $p=0.04$) and BAT glucose uptake ($r=0.38$, $p=0.13$) and whole body insulin sensitivity ($r=0.66$, $p<0.0001$) at the baseline of the study. After weight loss, increase in SIRT1 correlated significantly with an increase in BAT glucose uptake ($r=0.68$, $p=0.004$).

Conclusion: Downregulation of SIRT1 expression in WAT in obesity correlates with higher liver fat and poorer whole body insulin sensitivity. SIRT1 levels in WAT increase during weight loss, together with an activation of BAT glucose uptake. This suggests that activation of SIRT1 expression in human WAT is of therapeutic potential, possibly through induction of BAT-like features in human WAT.

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Supported by: A.K.A., N.N., K.H.P., K.H.P., S.K., A.R.

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Dopaminergic effects on brown adipose tissue

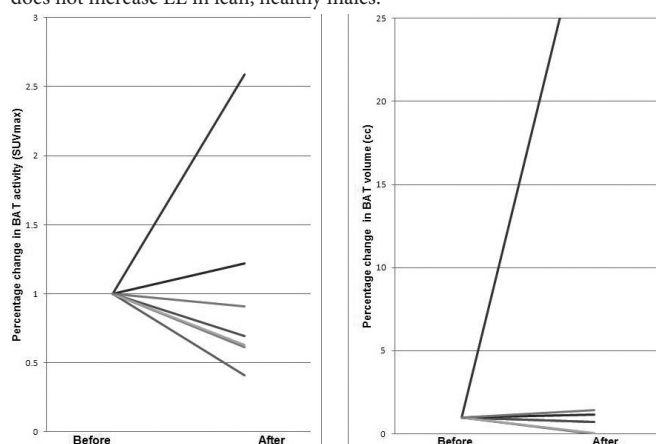
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Background and aims: Bromocriptine is a centrally acting dopamine receptor agonist that improves insulin sensitivity in obese subjects. Yet, no explanation has been found for this effect of bromocriptine. Brown adipose tissue (BAT), a tissue that converts calories into heat, might be involved in this process. Since the central sympathetic nervous system is the primary activator of BAT, we hypothesized that dopamine plays a role in the activation of BAT. Therefore, the aim of this study was to investigate the influence of bromocriptine on BAT activity in lean, healthy males.

Materials and methods: We included 8 lean (BMI 23[21–25] kg/m²), healthy Caucasian males (20.9[19–23] years). All subjects were studied before and after using bromocriptine (1st week 1,25mg/day, 2nd week 2,5mg/day in the evening) in a climate room at 21°C after an overnight fast. On these 2 study visits we measured metabolic BAT activity, defined as maximal standardized uptake value (SUVmax), using 18F-Fluorodeoxyglucose Positron Emission Tomography CT scans. Furthermore we investigated glucose metabolism with a 7 point oral glucose tolerance test, energy expenditure (EE) using indirect calorimetry, weight and body temperature. Subjects recorded their eating behavior in the 4 days before the study visits.

Results: The use of bromocriptine did not significantly alter metabolic BAT activity (SUVmax before 11.97[4.3–15.8]; after 10.3[2.7–18.2]), EE (before 2103 Kcal/day [1340–2486]; after 1915 [1784–2437]), body temperature (before 36.0 °C [35.6–36.4]; after 36.2 [36.0–36.7]) or weight (80 kg[72.1–82.2]; after 80 [72.1–81.8]). Unexpectedly, subjects became significantly less insulin sensitive after bromocriptine use. The area under the curve for glucose increased (before 652 [539–752]; after 857 [772–992] (p=0.02)). But the area under the curve for insulin also increased (before 27x103 [26x103–37x103]; after 44x103 [41x103–65x103] (p=0.03)). There were no changes in diet between the 2 measurements that could explain the change in insulin sensitivity.

Conclusion: We conclude that bromocriptine does not activate BAT and does not increase EE in lean, healthy males.



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Taurine treatment prevents obesity and diabetes through normalisation in circadian rhythms

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Background and aims: Obesity and type 2 diabetes are associated with disruption of circadian rhythms. It has been suggested that several nutrients are potential candidates to correct alterations in circadian rhythms and prevent these diseases. More over the aminoacid taurine has positive effects on metabolic dysfunction in obesity and diabetes in animal models of high fat diet. However, there is no study whether taurine can regulate circadian rhythms in mice. Therefore, we aim to study whether long term taurine treatment can ameliorate disturbances in circadian rhythms caused by high fat diet feeding

Materials and methods: Male C57BL/6 mice were divided in 4 groups. Control (C): mice fed with chow and Control+ taurine (C+T): mice fed with chow and 2.0% (w:v) of taurine in the drinking water. Obesity and diabetes were induced by HFD 45% of fat, and HFD + taurine group (HFD+T) treated with 2.0% (w:v) taurine in the drinking water. Glucose tolerance test and insulin tolerant test were performed in the last week of intervention. After 10 weeks of taurine treatment mice were sacrificed at different times of the day (6:00, 12:00, 18:00, 24:00). Plasma insulin levels were measured by ELISA. The expressions of clock genes in isolated pancreatic islets were measured by RT-PCR.

Results: Mice treated with HFD increased food intake (p<0.001) and insulin levels (p<0.05) during the day and night time compared to chow fed mice. HFD group increased body weight (p<0.01) and visceral fat (p<0.05). HFD group disrupted the circadian pattern of insulin and exhibited higher insulin levels throughout the 24h. HFD+T treatment prevented the increase in food intake and plasma insulin during the day and night. HFD+T group had a decrease in body weight (p<0.02) and visceral fat (p<0.05). Taurine prevents the impairment in glucose tolerance and prevents insulin resistance, decreased plasma insulin levels and restored the oscillatory pattern of insulin in mice treated with HFD. Expression of clock genes in isolated pancreatic islets was measured. Rev-erb alpha, Bmal1 and Per1 were downregulated by HFD and taurine prevents this effect normalizing the expression of Per 1 at 18h (p<0.01).

Conclusion: Taurine can prevent disturbances of circadian rhythm in food intake and insulin that was disrupted by HFD, and improves glucose metabolism in HFD treated mice. These results suggest that these beneficial effects of taurine could be a potential candidate to prevent obesity and diabetes.

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Effects of resveratrol in experimentally induced endotoxaemia in mice

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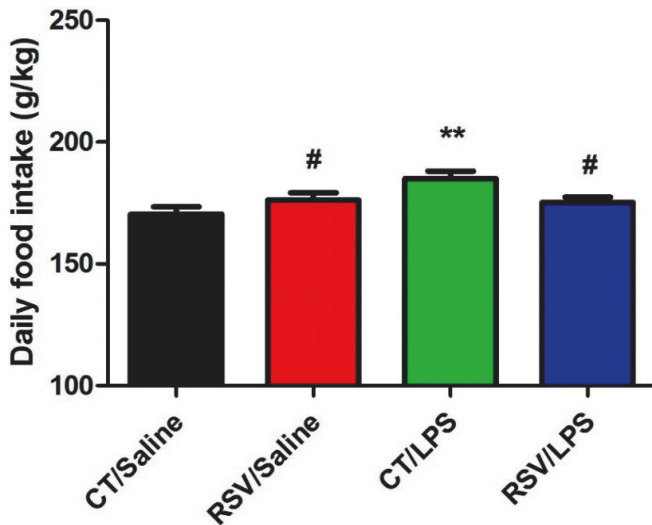
Background and aims: Obesity is rapidly increasing in prevalence worldwide followed by a number of comorbidities such as cardiovascular diseases and type 2 diabetes. Obesity is often characterized by a chronic low-grade inflammation, which is believed to induce insulin resistance and ultimately diabetes. The triggering factor of this inflammatory state is unknown but lipopolysaccharide (LPS) originating from the gut microbiota has been suggested as a possible explanation hereof. Resveratrol is a naturally occurring polyphenol found in especially red wine, which has potential anti-inflammatory and anti-diabetic effects. RSV activates the intracellular deacetylase sirtuin-1 (SIRT1) and inhibits nuclear factor κB (NF-κB) mediated inflammation. SIRT1 activation has been shown to mimic many of the positive effects seen of calorie restriction on metabolism.

Materials and methods: C57BL/6 mice were surgically implanted with miniosmotic pumps subcutaneously infusing LPS (600µg/kg/day) or saline. The mice had free access to water and normal diet (CT) or a RSV-modified diet (4g/kg) for the duration of 30 days. Body weight and food consumption were measured weekly. Glucose metabolism was evaluated by oral and intraperito-

neal glucose tolerance tests (OGTT/IPGTT). Also, tissue and blood samples were collected for biochemical analyses and gene expression data.

Results: LPS-infused mice had an increased daily food intake compared to controls (185.1 ± 3.0 vs. 170.5 ± 2.9 g/kg, $P < 0.01$), which was reversed by RSV (175.2 ± 2.2 g/kg, $P < 0.05$). Despite this, there were no differences in average body weight gain after 28 days of treatment between the groups. Fasting glucose was reduced by RSV after 28 days of treatment (7.7 ± 0.2 vs. 8.9 ± 0.4 mM, $P < 0.01$). During an OGTT, LPS-treated mice had a reduced hyperglycemia 15 min after glucose administration compared to control animals (14.9 ± 0.6 vs. 17.5 ± 0.8 mM, $P < 0.05$). RSV had no effect on the glucose metabolism during OGTT. Neither RSV nor LPS had any significant effect on transcriptional expression of glucagon-like peptide-1 (GLP-1), peptide YY (PYY), glucose-dependent insulinotropic peptide (GIP), neurotensin, TNF- α , prohormone convertase 1/3 (PC1/3) or dipeptidyl peptidase-4 (DPP4) in the ileum of the small intestine.

Conclusion: LPS-infusion for 28 days induced hyperphagia without affecting the body weight. RSV reversed this LPS-induced hyperphagia. To our surprise, LPS-treated mice did not show a worsening in glucose clearance as has earlier been suggested. Actually, LPS-treated mice had a small reduction in hyperglycemia 15 min following oral glucose challenge.



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T-bet regulation of the gut microbiota impacts insulin sensitivity

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Background and aims: The gut microbiota plays a role in energy harvest, storage, and expenditure. Its composition differs in lean and obese humans and animals and transfer of microbiota from conventionally-raised to germ-free mice increases body fat content and insulin resistance. The immune system has co-evolved with the gut microbiota. We have recently highlighted the role of the immune cell transcription factor T-bet as a metabolic regulator uncoupling adiposity from insulin resistance. We hypothesised that the enhanced insulin sensitivity of T-bet (Tbx21) deficient mice was associated with an altered gut microbiota composition.

Materials and methods: Microbiota composition of T-bet deficient and control wild-type (WT) mice was analysed by 16S-pyrosequencing. Subsequent analyses of colonic metabolites (short chain fatty acids, SCFA) were assessed by gas chromatography-mass spectrometry. The impact of genotype on adiposity and glucose homeostasis was determined by glucose and insulin tolerance testing following microbiota transfer to antibiotic-treated mice.

Results: T-bet deficient mice showed a decrease in the Firmicutes and Deferribacteres phyla and a significant increase in the Bacteroidetes and TM7 phyla compared to wild-type mice. Furthermore, they exhibited an increase in the proportion of Clostridium species together with an increase in butyrate production (217.6 ± 30.6 vs 336.6 ± 32.9 μ g/g feces, WT vs T-bet^{-/-}, $p=0.02$).

Transfer of T-bet deficient microbiota to microbiota-depleted WT hosts improved insulin sensitivity (256.6 ± 7.2 vs 221.0 ± 8.8 , WT vs T-bet^{-/-} area under the curve insulin tolerance test, $p=0.02$). This was accompanied by an increase in SCFA production, especially butyrate (216.3 ± 76.0 vs 1581.1 ± 636.8 μ g/g feces, WT vs T-bet^{-/-} microbiota, $p=0.02$).

Conclusion: Our results suggest that deficiency of the immune cell transcription factor, T-bet is associated with an altered gut microbiota composition that can enhance systemic insulin sensitivity.

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TIP27 increases insulin sensitivity through PI3-kinase/Akt pathway and regulates glucose homeostasis in mice

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Background and aims: TIP27 (Juxtaposed with another zinc finger protein1) is a 27kDa transcription factor containing three putative zinc finger motifs and its expression is associated with diabetes mellitus and prostate cancer. However, little is known about its role in regulation of metabolism. In this study, we investigated the effects of TIP27 overexpression on glucose homeostasis and insulin signaling pathway in high fat diet (HFD)-fed TIP27 transgenic (Tg) mice and diabetic db/db mice.

Materials and methods: Mainly, we use TIP27-Tg mice as research object. Also, we established a gain-of-function model of TIP27 in db/db mice using an adenovirus-mediated cDNA. Using these models, we assessed the effects of TIP27 overexpression in both TIP27-Tg mice and db/db mice on glucose metabolism and changes in insulin sensitivity during glucose tolerance tests (GTT) and insulin tolerance tests (ITT). Hyperinsulinemic-euglycemic clamp experiments were performed in Tip27-Tg mice. Glucose rates of appearance (GRa) were determined with 3-[3H] glucose. Whole body GRa and glucose uptake (GRd) were calculated using the non-steady-state equation. mRNA and protein expressions were measured by qRT-PCR and Western blot, respectively.

Results: We showed that TIP27 overexpression in both TIP27-Tg mice and db/db mice led to reduced total cholesterol, fasting plasma insulin levels, enhanced glucose tolerance and insulin sensitivity. Hyperinsulinemic-euglycemic clamp experiments also demonstrated that TIP27 overexpression in TIP27-Tg mice enhanced insulin sensitivity. In addition, the expression levels of PEPCK and Glucose-6-phosphatase (G-6-Pase) mRNA as well as proteins were significantly decreased in TIP27-Tg mice, whereas the phosphorylations of insulin-receptor (IR), IRS-1, Adenosine Monophosphate Activated Protein Kinase (AMPK) and Akt were significantly increased in the insulin target tissues. Finally, inhibition of phosphatidylinositol 3-kinase (PI3-kinase)/Akt signaling by LY294002, a PI3-Kinase inhibitor, abolished the suppressive effect of TIP27 overexpression on PEPCK and G-6-Pase expression.

Conclusion: TIP27 plays an important role in glucose homeostasis by the regulation of hepatic glucose metabolism and insulin sensitivity and this regulation requires activation of the PI3-kinase/Akt pathway.

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LEOPARD syndrome-associated SHP2 mutation confers leanness and protection from diet-induced obesity

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Background and aims: LEOPARD syndrome (LS) is a rare autosomal dominant disorder associating various developmental defects notably cardiopathies, dysmorphism and short stature. It is mainly caused by mutations of the PTPN11 gene that catalytically inactivate the ubiquitous tyrosine phosphatase SHP2. SHP2 plays essential roles in transducing canonical signaling pathways (PI3K, MAPK) in response to a wide range of growth factors

and hormones. Recent studies using targeted invalidation of SHP2 in various tissues and organs pinpointed key roles for SHP2 in regulating energy metabolism. However, the metabolic outcomes of LS mutations have never been examined. Therefore, we aimed to evaluate the metabolic status associated with LS using an original LS mouse model expressing one of the most common LS-associated SHP2 mutations. Beyond LS pathophysiology, since this mutation results in systemic partial inhibition of SHP2, this study also allowed assessing its role in the regulation of whole body metabolism.

Materials and methods: We performed a comprehensive analysis of the metabolic impact of LS-associated SHP2 mutations, by combining an extensive metabolic exploration of LS mice and of their WT littermates (body composition, glucose/insulin tolerance, energy expenditure,...) with functional analysis in cellulo. Animals were handled in accordance with the principles and guidelines established by the local Ethics Committee.

Results: Our results reveal that LS mice gain less weight than their WT littermates ($p < 0.01$), which correlates with a strong reduction of adiposity (2/3 less at 28 weeks of age, $p < 0.001$) despite similar food intake between genotypes. We provide evidences that LS mutant expression impairs adipogenesis and triggers energy expenditure ($p < 0.001$), two features that can contribute to the reduced adiposity of LS mice. This lean phenotype is associated with improved glucose and insulin tolerances ($p < 0.05$). Consistently, we show that expression of LS mutants in a cell line induces a hyperphosphorylation of both Akt and Erk1/2 upon insulin stimulation ($p < 0.05$), suggesting enhanced insulin sensitivity. Interestingly, chronic treatment of LS mice with low doses of MEK inhibitor results in weight and adiposity gains ($p < 0.05$). Moreover, LS mice appear to be resistant to high fat diet-induced obesity and protected from associated disorders as insulin resistance (HOMA-IR WT 10.31 ± 2.60 vs LS: 1.25 ± 0.35 , $p < 0.05$) or ectopic triglycerides deposits in the liver (WT: 140 ± 15 vs LS: 73 ± 18 mg/g liver, $p < 0.05$) or the muscle (WT: 0.22 ± 0.02 vs LS: 0.15 ± 0.03 g/g prot, $p < 0.05$). Importantly, preliminary data in a French cohort of LS patients show that most of them have lower-than-average body mass index, which is associated, for tested patients, with reduced adiposity.

Conclusion: Altogether, our study demonstrates, for the first time, that LS status is associated with a metabolic benefit in vivo. In a more general context, our findings also highlight a key role for SHP2 in the regulation of whole body energy homeostasis and suggest that partial SHP2 inactivation could be protective towards the development of obesity and associated disorders.

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Transient reduction of hyperinsulinaemia in growing female *Ins1^{-/-}:Ins2^{+/-}* mice protects against diet-induced obesity throughout adulthood

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Background and aims: There is clinical and experimental evidence that hyperinsulinemia can precede and perhaps cause diet-induced obesity. We have reported that preventing lifelong hyperinsulinemia by reducing the dosage of *Insulin 1* in *Insulin 2* null male mice (*Ins1^{-/-}:Ins2^{-/-}*) blocks diet-induced obesity. However, this model did not address effects of the ancestral *Ins2* gene (analogous to human *INSULIN*), nor whether protection against obesity would persist without sustained repression of hyperinsulinemia. In the present study, we examined effects of reducing the ancestral *Ins2* gene, in the absence of rodent-specific *Ins1*. Temporary repression of hyperinsulinemia in high fat-fed *Ins1^{-/-}:Ins2^{+/-}* females allowed us to test the hypothesis that a transient reduction of insulin secretion in young mice can provide long-term protection against obesity.

Materials and methods: Female *Ins1^{-/-}:Ins2^{+/-}* mice and *Ins1^{-/-}:Ins2^{+/+}* littermate controls were put on a high fat diet (HFD) or chow diet at weaning ($n = 31$ –34). Insulin secretion, body mass, and body composition (DEXA) were tracked for at least 1 year, in addition to parameters of metabolic health including blood glucose response to glucose or insulin injections and levels of several hormones.

Results: Female *Ins1^{-/-}:Ins2^{+/-}* mice had lower circulating insulin as early as eight weeks of age, compared to *Ins1^{-/-}:Ins2^{+/+}* littermates ($p < 0.05$). At 27 weeks, all HFD-fed females had elevated basal insulin levels, but this was reduced by nearly 50% in *Ins1^{-/-}:Ins2^{+/-}* females (*Ins1^{-/-}:Ins2^{+/+}* 1.17 ± 0.23 ng/mL; *Ins1^{-/-}:Ins2^{+/-}* 0.62 ± 0.14 ng/mL; $p < 0.05$). Of note, reduced circulating insulin in *Ins1^{-/-}:Ins2^{+/-}* mice did not elevate blood glucose. Attenuated hyperinsulinemia in HFD-fed *Ins1^{-/-}:Ins2^{+/-}* females corresponded with reduced weight gain and fat mass expansion, although this divergence could not be explained by differences in energy expenditure or food intake in 17 week-

old mice. Interestingly, while *Ins1^{-/-}:Ins2^{+/-}* females on a HFD reached an equivalent degree of hyperinsulinemia as HFD-fed *Ins1^{-/-}:Ins2^{+/+}* littermates by one year (*Ins1^{-/-}:Ins2^{+/+}* 1.24 ± 0.13 ng/mL; *Ins1^{-/-}:Ins2^{+/-}* 1.21 ± 0.23 ng/mL; $p = 0.8$), their body mass never matched the more obese *Ins1^{-/-}:Ins2^{+/+}* mice (*Ins1^{-/-}:Ins2^{+/+}* 36.4 ± 1.2 g; *Ins1^{-/-}:Ins2^{+/-}* 30.9 ± 0.9 ng/mL; $p < 0.5$). Unlike control *Ins1^{-/-}:Ins2^{+/+}* females, 40 week-old HFD-fed *Ins1^{-/-}:Ins2^{+/-}* females did not have high leptin levels (*Ins1^{-/-}:Ins2^{+/+}* 6.3 ± 2.2 ng/mL; *Ins1^{-/-}:Ins2^{+/-}* 1.1 ± 0.4 ng/mL; $p < 0.5$); other hormones such as gastric inhibitory polypeptide were similarly elevated in all HFD-fed mice. Reduced insulin sensitivity and a decline in glucose tolerance were evident in all HFD-fed mice by 52 weeks, despite decreased weight gain in *Ins1^{-/-}:Ins2^{+/-}* mice.

Conclusion: Our results demonstrate that elevated insulin levels in growing female mice are associated with increased body mass and fat mass, and the maintenance of obesity throughout life. Attenuation of hyperinsulinemia during the first six months of murine life provides a long-lasting protection from obesity. Importantly, this protection persists despite a late-onset emergence of pronounced hyperinsulinemia in high fat-fed *Ins1^{-/-}:Ins2^{+/-}* female mice. These findings suggest that suppressing elevated insulin levels during the growth period of adolescence and young adulthood can have far-reaching consequences on the obese phenotype of fully grown adults.

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Partial pancreatic *Ins2* gene deletion reverses diet-induced obesity in adult male mice

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Background and aims: Obesity is an epidemic affecting more than 500 million people worldwide. Furthermore, it is an important risk factor to several diseases, including heart disease, diabetes, stroke and cancer. Obesity is commonly associated with basal hyperinsulinemia and insulin resistance, but the cause and effect relationship between obesity, hyperinsulinemia, insulin resistance and type 2 diabetes remains to be fully elucidated. Some evidence suggests a causal role for hyperinsulinemia in obesity, independently of insulin resistance. Drugs that block hyperinsulinemia have been reported to cause weight loss in humans, but this has remained controversial and it has been suggested that such drugs work independently of insulin. We have recently reported that life-long suppression of basal hyperinsulinemia in male *Ins1^{+/-}:Ins2^{-/-}* mice resulted in protection from diet-induced obesity via mechanisms that included the reprogramming of white adipose tissue to express Ucp1. While these results provided the first evidence hyperinsulinemia can play a causal role in mammalian obesity, they were unable to distinguish between acute and chronic effects of insulin and therefore could not answer whether insulin reduction in adult, obese mice would result in weight loss. To address this critical therapeutic question, we developed a mouse model with acute 50% deletion of the insulin 2 gene.

Materials and methods: We generated an inducible, 50% beta-cell specific insulin gene knockout mouse model (*Pdx1CreER:Ins1^{-/-}:Ins2f/+*) to test the hypothesis that diet-induced obesity can be lowered by simply reducing pancreatic insulin production, which would indicate that long-term developmental reprogramming of the insulin system is not required to protect mammals from obesity. Control chow (25% fat) and high fat (58% fat) diets were initiated in both female and male mice at 6 weeks of age. Following 12 weeks on the respective diets, test and littermate control mice were injected with tamoxifen (or vehicle) resulting in the inducible loss of one *Ins2* allele in test mice.

Results: High fat diet-induced obesity was observed by 12 weeks of diet when compared to littermate control mice fed a control chow diet, and was especially pronounced in males. Remarkably, inducible reduction of pancreatic insulin 2 gene dosage in tamoxifen-injected *Pdx1CreER:Ins1^{-/-}:Ins2f/+* male mice resulted in a 5% rapid weight loss to the levels seen in mice on control diets within 3 weeks. This effect was also only seen in mice fed a high fat diet as body mass did not differ between mice fed a chow diet following tamoxifen injection. The weight loss observed in the HFD male mice, which was due to reduced fat mass, was not a result of hyperglycaemia, as fasted blood glucose did not differ from control male mice on the HFD (control, 8.4 ± 0.2 ; test, 8.4 ± 1.3 , $p = 0.99$). Gene expression patterns were assessed in multiple tissues to provide molecular clues to the rapid mechanisms of weight loss subsequent to the abrogation of diet-induced hyperinsulinemia. Energy expenditure and related parameters were assessed using metabolic cages.

Conclusion: Together, these data provide the first evidence in any organism that obesity can be reversed by acutely reducing the production and circulation of insulin. Our results have profound implications for nutritional guidelines and therapeutic efforts to combat obesity.

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BACE2 suppression ameliorates beta cell dysfunction induced by human islet amyloid polypeptide (IAPP) overexpression

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Background and aims: BACE2 (β -site APP-cleaving enzyme 2) is a β -protease that has been found in the brain, where it is thought to play a role in the development of Alzheimer's disease (AD). It has also been localized in the pancreas, where it seems to play a physiological role since BACE2-deficient mice elicit better glucose tolerance than control littermates. Amyloidogenic diseases, including AD and type 2 diabetes (T2D), have been reported to share the accumulation of abnormally folded and insoluble proteins that interfere with cell function. In the case of T2D, amylin (IAPP) deposits have been shown to be a key feature of the disease. The aim of the present study was to investigate the effect of BACE2 modulation on β -cell alterations induced by IAPP overexpression.

Materials and methods: Heterozygous-hIAPP mice, BACE2-KO mice and their respective controls were used to analyze their phenotype after 16 weeks with high-fat diet (HFD) feeding. Afterward, these two models were crossed in order to analyze the impact of BACE2 suppression on β -cell alterations observed in Tg-hIAPP mice. Insulin tolerance test (ITT) and glucose tolerance test (GTT) were performed to evaluate the metabolic phenotype, and the area under the curve (AUC) of the GTT was calculated as a measure of glucose homeostasis. The ability to secrete insulin in response to glucose (GSIS) was quantified with an ELISA kit. Proliferation was analyzed by Ki67 immunostaining and β -cell mass by insulin immunostaining.

Results: The GTT of Tg-hIAPP mice after 16 weeks of regular chow diet revealed glucose intolerance with respect to the wild type animals. These animals showed a 1.3-fold increase ($p<0.05$) in β -cell mass, however, the secretory response of insulin was reduced after the glucose challenge (25% decrease $p<0.05$, vs. control littermates). 16 weeks of HFD feeding induced insulin resistance and glucose intolerance, both in Tg-hIAPP and wild type animals. On the other hand, BACE2-KO mice showed better glucose homeostasis than their wild type counterparts, which correlates with a 50% enhancement of insulin secretory response to glucose injection ($p<0.05$) and an increase in β -cell mass and β -cell proliferation. Moreover, BACE2-KO mice fed with a HFD showed an 18% reduction in body weight ($p<0.05$) and better glucose homeostasis than wild type animals (28% decrease in AUC of GTT), indicating that deletion of BACE2 protects against HFD. The crossed animals (Tg-hIAPPxBACE2-KO) presented a significant improvement in glucose tolerance as compared to Tg-hIAPP mice (18% decrease in AUC). This improvement was due to an enhanced insulin secretory response to glucose load (15% increase vs. Tg-hIAPP mice, $p<0.05$), indicating a potential effect of BACE2 deletion on β -cell function.

Conclusion: The inhibition of BACE2 compensates glucose tolerance defects induced by hIAPP overexpression in the β -cell. Thus, targeting BACE2 may represent a good therapeutic strategy to improve β -cell function in T2D.

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Altered energy metabolism in vivo and in vitro with amyloid beta42 administration

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Background and aims: Amyloid β_{42} ($A\beta_{42}$) is a peptide associated with the onset of Alzheimer's Disease (AD) and impairment of neuronal metabolism. $A\beta_{42}$ is also peripherally secreted and increased levels of circulating $A\beta_{42}$ are found in obese and diabetic patients. It is unknown whether circulating $A\beta_{42}$ contributes to altered glucose metabolism or insulin action in Type 2 diabetes (T2D). The aim of our study was to determine whether administration of $A\beta_{42}$ alters glucose metabolism of insulin sensitive cells *in vitro*, and whole body metabolism *in vivo*.

Materials and methods: Basal and insulin stimulated glucose uptake and glucose production was measured in 3T3-L1 adipocytes and FAO hepatocytes respectively after treatment with either monomeric (m) $A\beta_{42}$ or aggregated (a) $A\beta_{42}$ at various concentrations (0, 100, 200, 300nM) for 48h; scrambled (scr) $A\beta_{42}$ was used as control. For *in vivo* studies, 6 week old, male C57Bl/6J mice ($n=10$ per group) were injected i.p. with recombinant m $A\beta_{42}$ or control scr $A\beta_{42}$ for five weeks (1 μ g/day). Bodyweight and composition, indirect calorimetry and glucose and insulin tolerance were measured before and after treatment to determine the effect of m $A\beta_{42}$ administration on these measures. The clearance rate of m $A\beta_{42}$ from plasma was determined from mice that had been treated for two weeks, with plasma samples collected at various time points before (0) and post i.p. injection (1, 15, 30, 60min, 2, 4, 8, 24h) of m $A\beta_{42}$ and scr $A\beta_{42}$.

Results: Monomeric $A\beta_{42}$ increased both basal ($p<0.001$) and insulin ($p<0.01$) suppressed glucose production at higher doses (200; 300nM) in FAO hepatocytes compared with respective controls, while a $A\beta_{42}$ had no effect. In 3T3-L1 adipocytes, m $A\beta_{42}$ as well as a $A\beta_{42}$ impaired basal ($p<0.001$) and insulin stimulated ($p<0.01$) glucose uptake at higher doses (200; 300nM) compared with respective controls. *In vivo* administration of m $A\beta_{42}$ increased basal circulating levels of m $A\beta_{42}$ compared with scr $A\beta_{42}$ treated mice (3.4ng/ μ l vs 0.8ng/ μ l; $p<0.05$) and also total m $A\beta_{42}$ exposure over 24 hours in m $A\beta_{42}$ treated mice ($p<0.05$). Administration of m $A\beta_{42}$ had no effect on bodyweight, body composition or food intake compared with control mice and did not induce glucose or insulin intolerance. However, m $A\beta_{42}$ treated mice displayed increased fasting glucose compared with control mice. There was no change in oxygen consumption between the two treatments, but administration of m $A\beta_{42}$ resulted in a decrease in RER compared with control mice.

Conclusion: Our *in vitro* data suggest that primarily m $A\beta_{42}$ impairs insulin dependent and independent glucose metabolism in multiple cell types. In contrast to recently published studies, our *in vivo* data show that treatment with physiological levels of m $A\beta_{42}$ had no impact on glucose or insulin tolerance, despite elevated fasting glucose levels and a substrate shift towards lipid oxidation compared with control animals. These data suggest that not only is $A\beta_{42}$ involved in the pathology of AD, but that $A\beta_{42}$ could also be involved in the dysregulation of metabolism in obesity and T2D via insulin dependent and/or independent mechanisms.

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Transgenerational phenotypic changes in selectively bred mice for different susceptibilities to diet-induced glucose intolerance

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Background and aims: Gene-environment interactions (GEI) play a crucial role in the development of type 2 diabetes. To establish novel rodent models that can mimic the GEI, we have performed a selective breeding of mice. In brief, using 3 inbred strains (C57BL/6, C3H, and AKR) as background, mice exhibiting superior and inferior glucose tolerance after high fat diet (HFD) feeding have been bred repetitively over 20 generations to establish 2 distinct mouse lines with different susceptibilities (resistant and prone) to HFD-induced glucose intolerance, and designated SDG-R and SDG-P, respectively. Here, we analyzed transgenerational changes of metabolic phenotypes in the selective breeding of SDG-R and SDG-P to explore the hereditary predisposition to diet-induced glucose intolerance.

Materials and methods: All mice were fed with HFD (32% energy from fat) for 5 weeks (5–10 weeks of age) for the selective breeding of SDG-R and SDG-P. During the 5–20th generations, HFD intake was monitored every week, body weight was measured at the end of HFD feeding, and OGTT (2 g glucose/kg body weight) was performed within 1 week after the end of the HFD feeding period. In the OGTT, mice of both sexes showing lower and higher blood glucose levels at 120 min ($BG_{120\text{ min}}$) were selected to breed the next generations of SDG-R and SDG-P, respectively.

Results: The selection index $BG_{120\text{ min}}$ was markedly increased as the generations proceeded in SDG-P mice of both sexes (male, $p<0.0001$; female, $p<0.0001$; Jonckheere-Terpstra trend test). Although $BG_{120\text{ min}}$ was also elevated slightly in SDG-R mice (m, $p=0.0016$; f, $p=0.0091$), distinct differences were seen in $BG_{120\text{ min}}$ between the 2 lines at the 20th generation [SDG-R vs. SDG-P (mean \pm SEM mmol/l, *t*-test): m, 5.60 ± 0.23 vs. 19.9 ± 1.13 ($p<0.0001$); f, 4.34 ± 0.17 vs. 9.60 ± 0.62 ($p<0.0001$)]. In addition, fasting blood glucose levels (FBG) were increased in SDG-P mice of both sexes (m, $p<0.0001$; f, $p<0.0001$), whereas it was not altered in male ($p=0.553$) and gradually de-

creased in female ($p<0.0001$) SDG-R mice. Post-HFD body weight was increased in SDG-P mice of both sexes (m, $p<0.0001$; f, $p<0.0001$) as the generations proceeded, whereas it was not altered in male ($p=0.709$) and decreased slightly in female ($p=0.0006$) SDG-R mice. Despite the increasing trend of body weight in SDG-P mice, HFD intake was not altered in male ($p=0.084$) and even decreased in female ($p=0.0002$) SDG-P mice. In SDG-R mice, HFD intake was significantly decreased in both sexes (m, $p<0.0001$; f, $p<0.0001$). Linear regression analysis revealed that differences in the average values of BG120 min, FBG, post-HFD body weight, and HFD intake between the 2 lines became more evident as the generations proceeded.

Conclusion: Even though BG_{120 min} was the sole criterion for the selective breeding, SDG-P mice became more susceptible to HFD-induced obesity as well as glucose intolerance. In SDG-P mice, despite the increasing trend in body weight, HFD intake was not increased as generations proceeded, suggesting that SDG-P mice had acquired a “thrifty metabolism” through the selective breeding. On the other hand, SDG-R mice had reduced HFD intake as the generations proceeded, suggesting that SDG-R mice had adapted feeding regulation to maintain glucose tolerance under HFD. These differences may determine the distinct susceptibilities to diet-induced metabolic impairments in the 2 lines of mice.

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PS 050 Adipose tissue function: animal models

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Adipose tissue mRNA expression of WHR-associated genes exhibit inter-depot variability and correlates with fat distribution

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Background and aims: Body fat distribution (FD) is one of the main predictors of obesity associated complications. There is good evidence that fat distribution is controlled by genetic factors. Genome-wide association studies revealed novel loci associated with waist to hip ratio (WHR). Here, we hypothesized that genes within these loci exhibit fat depot-specific mRNA expression, which correlates with obesity-related traits. Furthermore, we conducted expression quantitative trait loci (eQTL) analyses to test whether WHR-associated variants may affect the mRNA expression of the corresponding genes in adipose tissue (AT).

Materials and methods: By using qRT-PCR, we measured mRNA levels of *LYPLAL1*, *ADAMTS9*, *VEGFA*, *CPEB4*, *RSPO3*, *ITPR2*, *SSPN*, *LY86* in paired human samples of visceral and subcutaneous AT from 570 individuals with detailed metabolic testing. Previously reported single nucleotide polymorphisms (SNPs) associated with WHR were genotyped in all subjects for subsequent eQTL analyses. Since one of the SNPs (rs6795735) maps within the *ADAMTS9-Antisense RNA 2* (*ADAMTS9-AS2*), we measured also the mRNA expression of *ADAMTS9-AS2*.

Results: All tested genes exhibited significantly higher mRNA levels in visceral relative to subcutaneous AT. In addition, the mRNA levels correlated with WHR (*ADAMTS9*, *VEGFA*, *SSPN*, *LY86*), % body fat (*ADAMTS9*, *VEGFA*, *CPEB4*, *LYPLAL1*) and BMI (*ADAMTS9*, *CPEB4*). None of the SNPs was significantly associated with the mRNA levels or metabolic traits including WHR or BMI, which is likely to be attributed to the small sample size, and so lack of statistical power. However noteworthy, we observed a strong correlation between the mRNA expression of *ADAMTS9-AS2* and *ADAMTS9*, suggesting that the previously reported association of the WHR-associated SNP rs6795735 might be mediated by its effects on *ADAMTS9-AS2*.

Conclusion: Our data including the inter-depot variability of mRNA expression and its strong correlation with metabolic traits suggest a role of genes within the WHR-associated loci in the regulation of fat distribution. In particular *ADAMTS9*, *VEGFA* and *CPEB4* are promising candidates for functional analysis of their role in adipocyte biology. This work is currently going on.

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GLP-1 receptor agonist-induced lipolysis in adipocyte is mediated by SIRT1

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Background and aims: Accumulated evidence suggests GLP-1 and its mimetics exert their beneficial effects in extra-pancreatic organs. Here, we found that GLP-1 receptor agonist exenatide might reduce visceral fat by increasing lipolysis in adipocytes, and we also explored the potential role of SIRT1 in this effect both in vivo and in vitro.

Materials and methods: In this study, 8 weeks male SIRT1 heterozygous knockout mice (SIRT1^{+/-}) in C57BL/6J gene background and their wild type littermates (WT) were used. After 12 weeks chow diet feeding or high-fat diet (HFD) challenge, animals were randomly divided into the following 5 groups: WT+chow diet, WT+HFD+saline, WT+HFD+exenatide, SIRT1^{+/-}+HFD+saline and SIRT1^{+/-}+HFD+exenatide. Mice were treated with intraperitoneal injection of exenatide (24 nmol/kg) or normal saline control daily for another 8 weeks. Systemic evaluations were carried out including body weight, FBG, ip GTT, ip ITT, blood lipids profile, and weight of epididymal fat. Quantitative RT-PCR and western blot were used to detect mRNA and protein expression in epididymal fat, respectively. In differentiated 3T3-L1 adipocytes, lentivirus vector expressing SIRT1 RNAi sequence was transfected

to knock down SIRT1, and transfected cells were then treated with exendin-4 for 24 h.

Results: The animal study showed that both body weight and epididymal fat weight were sharply decreased after exenatide treatment compared with saline control in WT+HFD group. However, the decrease in SIRT1^{+/+}+HFD group after exenatide treatment was much milder. While lipids profile showed the same change. Both RT-PCR and western blot results indicated that the expression of SIRT1 was up-regulated in WT+HFD group with exenatide treatment as compared with saline control; however, no significant difference was observed in SIRT1^{+/+}+HFD mice after exenatide treatment. In differentiated 3T3-L1 adipocytes, exendin-4 up regulated SIRT1 and p-AMPK expression in a dose-dependent way. Lipolytic related factors including p-ACC, ACC and ATGL were all induced by exendin-4. After knocking down SIRT1 with SIRT1 RNAi, these factors were down regulated dramatically even with exendin-4 treatment.

Conclusion: Our data demonstrated that GLP-1 receptor agonist exenatide could reduce visceral fat by inducing lipolysis in adipocytes, and this effect is mediated by SIRT1 both in vivo and in vitro. It might be a new mechanism for GLP-1 and its mimetics in the role of losing weight.

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Activation of adipose tissue cannabinoid receptors 1 (CB1R) alters antilipolytic action of insulin and increases lipolysis in mice

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Background and aims: Recent data indicate that activation of peripheral endocannabinoid system (ECS) in tissues such as liver, muscle or adipose tissue may directly influence carbohydrate and lipid metabolism. The existence of cannabinoid receptors 1 (CB1R) and ECS enzymatic machinery has been demonstrated in mature adipocytes, nevertheless studies regarding their role in lipogenesis and lipolysis control are often conflicting. This study was designed to examine the consequence of ECS activation by anandamide on lipolysis activity and related regulation pathways.

Materials and methods: Lipolysis activity was estimated measuring for 45 min plasma glycerol release in response to β 3-adrenergic receptor agonist (BRL37344) in wild type, DIO or CB1R^{-/-} mice after acute peripheral anandamide injection compared with vehicle. Additional in vitro experiments were conducted to test direct effects of anandamide on glycerol release and signaling pathways on adipose tissue explants exposed to various concentrations of insulin and norepinephrine.

Results: While anandamide alone had no remarkable effects on basal lipolysis, ECS activation potentiated the effect of BRL37344 on glycerol release in wild type mice. The effect of anandamide on stimulated lipolysis was strongly reduced in CB1R^{-/-} while it was amplified in obese animals whose adipose tissue CB1R mRNA expression was much higher than in lean mice. In control mice, the stimulatory effect of BRL37344 on glycerol release was totally counteracted by insulin injection (0,025UI/kg) while it was partially maintained in the presence of anandamide suggesting that ECS activation may be associated with an alteration of the inhibitory action of insulin on lipolysis. These findings were also observed in cultured explants exposed to anandamide in which inhibition of glycerol release by insulin was abrogated. Further, anandamide treatment increased protein levels of the active form of hormone-sensitive lipase and reduced level of Akt and Pi3K phosphorylation compared to control in accordance with a decrease in the activity of insulin-dependent signaling cascade.

Conclusion: All together, these data showed that activation of ECS in adipose tissue increases lipolysis by altering the antilipolytic action of insulin. This suggests that antagonism of CB1R may constitute a new strategy to limit ectopic fat deposition associated with obesity.

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Increased BMP4 improves insulin sensitivity and increases beige/brown adipogenesis in adult mice

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Background and aims: Bone Morphogenic Proteins (BMPs) belong to the transforming growth factor beta superfamily and regulate a wide range of developmental and cellular processes. BMP4 has been shown to be critical for adipose precursor cell commitment and differentiation to the white adipose lineage. However, recent data also suggest a role of BMP4 in beige/brown adipose cell differentiation. A previous study has examined the developmental effect of BMP4 in mice genetically engineered to overexpress BMP4 in the adipose tissue. However, to be a potential therapeutic target it is important to examine if BMP4 also is able to increase beige/brown adipogenesis in adult animals. This is further underscored by our recent findings that adipose precursor cells in obesity exhibit a resistance to the effects of BMP4. To examine the effect of BMP4, we injected adult mice with AAV8-BMP4 or AAV8-null virus vectors targeting the liver and, thus, increasing circulating BMP4 levels.

Materials and methods: Six weeks old male C57BL6/N mice received a retro-orbital injection of AAV8 expressing BMP4 or control (null) virus. The mice were fed a high fat diet (HFD) for 16 weeks and they underwent ip GTT, ip ITT and DEXA. Adipose tissue and muscle were taken for gene and protein analyses and immunohistochemistry was performed on paraffin-embedded, formalin fixed tissue samples. All reported results are statistically significant. Statistical analyses were performed using Students t-test or Mann-Whitney non-parametric U-test.

Results: HFD-BMP4 mice gained less weight than the HFD-null mice in spite of having the same food intake. They also had reduced total adipose tissue mass and smaller adipose cells. Furthermore, they had an improved glucose tolerance and were more insulin-sensitive than the HFD-null mice. In addition to the reduced fat mass they also had increased lean body mass. Both gene and protein expression of beige/brown adipose markers (TMEM26, CD137 and UCP1) were increased in the subcutaneous adipose tissue. Markers of mitochondrial biogenesis, like PGC1 α , were significantly increased in both skeletal muscles and the adipose tissue. Since obesity also has been shown to lead to increased adipose tissue fibrosis and BMP4 may exert an anti-fibrotic effect, we examined markers of this process. Interestingly, both α -SMA and CTGF mRNA levels were significantly reduced in the obese BMP4 mice.

Conclusion: We here demonstrate that increased circulating levels of BMP4 in obese and adult mice lead to clear positive phenotypic changes with reduced body fat, increased lean body mass together with increased insulin sensitivity and improved glucose tolerance. These effects are probably due to the marked induction of beige/brown adipose cells in the subcutaneous adipose tissue as well as increased mitochondrial biogenesis also seen in the skeletal muscles. BMP4 also reduced markers of adipose tissue fibrosis known to be increased in obesity.

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Glyoxalase-1 overexpression reduces body weight and adipokine expression, and improves insulin sensitivity in high-fat diet-induced obese mice

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Background and aims: The development of obesity, and especially the expansion of visceral adipose tissue (VAT), is associated with the development of inflammation and insulin resistance. We previously demonstrated an accumulation of advanced glycation endproducts (AGEs) in obese VAT, leading to dysregulated expression of adipokines. In addition, the increased AGE levels were associated with the development of insulin resistance. Based on previous work, we hypothesized that inhibition of the major AGE precursor methylglyoxal (MGO) attenuates the impaired adipokine profile and improves insulin sensitivity in obesity. Because MGO can be detoxified by the

glyoxalase-1 (GLO-1) enzyme, we used a GLO-1 overexpressing transgenic mouse model which was fed with a high-fat diet (HFD).

Materials and methods: After a run-in period of 4 weeks, both wild-type (WT) mice and GLO-1 transgenic mice (10 weeks old) were either fed with a low-fat diet (LFD, 10% kcal% fat) or a HFD (45% kcal% fat) for 15 weeks. In the last two weeks of the study we performed an intraperitoneal glucose tolerance test (ipGTT) and insulin tolerance test (ipITT). All mice were euthanized after 15 weeks of LFD or HFD and multiple organs were collected for ex vivo examination.

Results: Endogenous GLO-1 mRNA levels of the VAT were significantly lower in WT mice of the HFD group compared to the WT mice of the LFD group ($p < 0.01$). Activity of the GLO-1 enzyme was also reduced in the VAT of the HFD group ($p < 0.05$). Bodyweight gain was significantly increased after 15 weeks of HFD feeding compared to the LFD group (15.2 ± 4.7 vs 6.5 ± 2.2 gram, respectively; $p < 0.001$). Overexpression of GLO-1 in mice fed with the HFD inhibited the bodyweight gain significantly compared to the WT mice (10.5 ± 4.5 vs 15.2 ± 4.7 gram, respectively; $p < 0.05$), despite equal calorie intake (13.1 ± 2.0 vs 12.3 ± 1.6 kcal/day, respectively; $p = 0.30$). Furthermore, the VAT of the HFD group was characterized by a pro-inflammatory profile, as indicated by an upregulated mRNA expression of MCP-1, F4/80, MHC-II, CD11c and TNF α (all $p < 0.05$). Overexpression of GLO-1 in the HFD mice attenuated these increases in mRNA levels. Finally, HFD-induced insulin resistance was attenuated by GLO-1 overexpression, as assessed by the ipGTT ($p_{\text{trend}} < 0.001$) and the ipITT ($p_{\text{trend}} = 0.08$).

Conclusion: Our data demonstrate that overexpression of the GLO-1 enzyme is a potential mechanism to reduce body weight gain, carbonyl stress, adipose tissue inflammation and insulin resistance in HFD-associated obesity. Modulating the GLO-1 pathway may be a novel approach to prevent obesity-associated complications.

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Deletion of inhibitory G proteins protects from obesity

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Background and aims: Many hormones and other substances involved in the breakdown or storage of fatty acids signal via G protein coupled receptors (GPCRs). Upon binding of such ligands to the respective GPCRs the associated heterotrimeric G proteins are activated causing a dissociation of G α subunits from G $\beta\gamma$ dimers. The G α subfamily of inhibitory G proteins (G α_i) shows a sequence identity of up to 95% among the three isoforms G α_{i1} , G α_{i2} , and G α_{i3} . Due to this high homology it has been suggested that G α_i proteins serve the same functions, i.e. they are activated by a similar set of GPCRs and signal to an overlapping set of effectors. Interestingly, recent publications describe more and more individual tissue- and cell-specific functions for the different isoforms. In this study we aim to clarify the specific roles of G α_{i2} and G α_{i3} proteins for body weight gain in mouse models on normal (ND) and high-fat diet (HFD) conditions.

Materials and methods: In G α_{i2} - and G α_{i3} -deficient mice we analysed *in vitro* differentiation of adipocytes isolated from 4-6-week-old mice and *in vivo* body composition of mice fed a ND or HFD for 14 weeks by magnetic resonance imaging (MRI).

Results: G α_{i2} and G α_{i3} are both expressed in white adipose tissue. Interestingly, both G α_{i2} - and G α_{i3} -deficient mice show a lean phenotype on ND compared to their respective littermates. However, time occurrence of the weight differences is detectable in a genotype-dependent manner. G α_{i2} -deficient mice weigh less already at weaning, whereas G α_{i3} -deficient mice showed initially no differences. Magnetic resonance imaging revealed that 18-week-old G α_{i2} -deficient mice had significantly lower amounts of subcutaneous and visceral adipose tissue mass. In contrast, in G α_{i3} -deficient mice a reduced fat mass determined by MRI was first observed in 36-week old mice, which gained statistical significance at 56-weeks of age. In addition, G α_{i2} - and G α_{i3} -targeted mice were protected from remarkably weight gain on HFD. Since G α_{i2} -deficient mice have pronounced weight differences starting at an early stage, we analysed differentiation of white adipocytes *in vitro*. Significantly less Oil-red-O-positive G α_{i2} -deficient adipocytes were detectable, whereas G α_{i3} -deficient *in vitro* adipocyte differentiation was similar to wild-type cells.

Conclusion: We conclude that deletion of G α_{i2} and G α_{i3} protects from weight gain in an age-dependent manner and results in a leaner phenotype. Our data

suggest specific and independent roles of both isoforms in the differentiation of white adipocytes as well as in the regulation of adipose tissue mass.

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14-3-3 ζ controls mitotic clonal expansion and adipocyte differentiation via p27Kip1

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Background and aims: Obesity is a major risk factor for type 2 diabetes, but the molecular mechanisms underlying adipogenesis are not fully known. While undergoing mitotic clonal expansion, adipocytes acquire specific transcriptional networks to drive differentiation. Proper localization of transcriptional regulators depends on molecular adaptor proteins, such as those of the 14-3-3 protein family. Previously, we identified essential roles of 14-3-3 ζ in the regulation of adipogenesis *in vitro* and *in vivo*. Given its ability to regulate many transcription factors, we sought to examine the effects of 14-3-3 ζ knockdown on the adipogenic transcriptome.

Materials and methods: 3T3-L1 cells were treated with insulin, IBMX, and dexamethasone for 0, 24, or 48 hours to induce differentiation. Cells were transfected with siRNA targeting 14-3-3 ζ or a scrambled control (siCon). Libraries for RNA-Seq were analyzed with an Illumina Hi-Seq 2500 (20 million paired-end reads per sample). Differentially expressed genes ($p < 0.001$ cut-off) were subjected to Gene-set enrichment analysis (GSEA; FDR < 25%) to determine biological processes affected by differentiation or si14-3-3 ζ . Flow-cytometry was used to quantify the proportion of cells at stages of the cell cycle. Adipocyte differentiation was assessed by Oil Red-O staining and immunoblotting for Ppar γ .

Results: RNA sequencing revealed that >5000 genes were significantly altered ($p < 0.001$) during the first 48 hr of differentiation. Categorization of differentially expressed genes by GSEA showed negatively enriched gene sets ($p < 0.0001$) that were related to the cell cycle, fat cell differentiation, and regulation of transcription in 14-3-3 ζ -depleted cells. Induction of numerous genes associated with the cell cycle (GO:000749) and mitotic cell cycle phase transition (GO:0044772), such as Cdc25a, Cdk6, and Cdkn1b, were significantly impaired following 14-3-3 ζ knockdown. To further explore the effect of 14-3-3 ζ on the cell cycle, si14-3-3 ζ -transfected 3T3-L1 cells were induced to differentiate and subjected to flow cytometry at 0, 24, and 48 hours. Knockdown of 14-3-3 ζ prevented cells from entering S-phase (25% vs 8%, $p < 0.01$). P27Kip1 (encoded by Cdkn1b) regulates G1-S transition, and depletion of 14-3-3 ζ promoted a 4-fold increase ($p < 0.05$) in p27Kip1 in undifferentiated cells. Differentiation caused a gradual decrease in p27Kip1 levels, but its expression remained elevated in si14-3-3 ζ -transfected cells. To test if elevated p27Kip1 contributes to the defects in si14-3-3 ζ -mediated cell cycle progression and differentiation, 3T3-L1 cells were co-transfected with siRNA against 14-3-3 ζ and p27Kip1. No effects on adipogenesis were observed in siCon- or sip27Kip1-transfected cells; however, co-transfection of sip27Kip1 into 14-3-3 ζ -deficient cells restored adipogenesis.

Conclusion: Our results demonstrate a novel role for 14-3-3 ζ in the cell cycle, as 14-3-3 ζ negatively regulates p27Kip1 expression. Loss of 14-3-3 ζ induces premature cell cycle arrest and ultimately blocks adipogenesis. Despite broad changes in global gene expression of pre-adipocytes, reductions in p27Kip1 alone are sufficient to rescue the defect in differentiation in 14-3-3 ζ deficient cells. These findings suggest that modulating 14-3-3 ζ or p27Kip1 expression could represent novel therapeutic approaches for the treatment of obesity.

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Deleted in breast cancer 1 (DBC1) plays a bifunctional role in adipocyte differentiation and inflammation

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Background and aims: Deleted in breast cancer 1 (DBC1) is a pleiotropic nuclear protein that interacts and modulates the activity of several molecules, including Sirt1, NF- κ B and Rev-erba, which exert important roles in adipocyte differentiation. Here, we aimed to investigate the possible role of DBC1 in adipose tissue physiology and in adipocyte differentiation.

Materials and methods: In two independent cohorts [cohort 1, participants with different degree of obesity (n= 105) and cohort 2, morbidly obese participants (n=47)], DBC1 gene expression was investigated in adipose tissue according to obesity and insulin resistance. DBC1 mRNA and protein levels during adipocyte differentiation and the effects of DBC1 knockdown (using Dbc1-targeted and control shRNA lentiviral particles) in the early stage of this process were also evaluated.

Results: DBC1 mRNA was detected at substantial levels in both visceral (VAT) and subcutaneous (SAT) adipose tissue (mainly in the adipocyte fraction), decreased in obese subjects, and directly associated with the expression of lipogenic and lipolytic genes in two both independent cohorts. Strikingly, DBC1 was linked to TNF mRNA levels in obese and morbidly obese participants and the administration of macrophage conditioned medium resulted in increased DBC1 gene expression in adipocytes. In line with these findings, in the early stages of adipocyte differentiation, Dbc1 knockdown led to decreased lipid accumulation and adipogenic gene expression in parallel to the increase of Sirt1 and AMPK activity. On the other hand, Dbc1 gene knockdown also led to a significant reduction in the expression of inflammatory genes (Tnf, Il6, Stamp2, Lbp and Mcp1) at the end of adipocyte differentiation.

Conclusion: Altogether these findings suggest that DBC1 exerts a bifunctional role in adipocyte physiology, being essential for adipocyte development, but also involved in adipocyte inflammation in the late stage of adipocyte differentiation.

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Antenatal antipsychotic exposure induces multigenerational and sex-specific programming of body weight and diabetes susceptibility in adult mouse offspring

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Background and aims: Atypical antipsychotic medications have revolutionized the treatment of severe mental diseases, but their metabolic side effects have been pointed, including obesity and diabetes. Among them, Olanzapine use has been reported during pregnancy and breastfeeding, but so far no clinical trials assessed the safety of in utero olanzapine exposure to fetuses and infants, or long term health outcome. In this study we analysed the transgenerational impact on metabolic homeostasis of mouse fetal antipsychotic exposure throughout gestational period.

Materials and methods: At 7d gestation, pregnant F0 CD1 mice received olanzapine (olz4 or olz8: 4 or 8 mg/kg/d) or haloperidol (hld: 2 mg/kg/day) or vehicle (control) through mini-osmotic pump until they gave birth naturally. F1 female offspring exposed in utero to olz (mat-olz) and hld (mat-hld) were bred to produce F2 offspring. Offsprings were weight every 3 days until the age of 3 weeks then weekly until 3 months of age. Glucose metabolism was investigated by insulin tolerance test (ITT), oral glucose tolerance test (OGTT) and plasma insulin measurement at the age of 3 months and 1 year for F1 offsprings, and at the age of 3 months for F2. Part of the F1 offspring cohort was sacrificed at 3 months and 1 year to analyse body and tissue composition and measure biochemical and hormonal parameters.

Results: The litter size of F1 mice was not influenced by the maternal treatment. However birth weight of mat-antipsychotic offspring groups of both sexes was significantly decreased compared to mat-control (p<0.01), suggesting an antipsychotic-induced intrauterine growth retardation. Only few weeks after birth, the initial difference in weight between mat-hld, female mat-olz and control disappeared. However, decreased weight of male mat-olz persisted throughout life (p<0.05). The results of OGTT and insulin measurement performed at the age of 3 months on F1 offspring were not different between mat-olz, mat-hld and control both in males and females. However mat-olz offspring developed a significant insulinoreistance compared to mat-ctrl. At the age of 1 year, a sexual dimorphism was observed. Concerning F1 male mat-olz, no modification in glucose metabolism (OGTT, ITT) or in hormonal parameters (insulin, leptin, adiponectin) or body mass composition was observed despite persistent lower weight compared to control (p< 0.05). However at the same time, mat-olz female developed impaired glucose tolerance (olz 4 and olz 8 vs ctrl p<0.05), hyperinsulinemia (olz 8 vs ctrl p<0.05) associated with insulinoreistance (olz 4vs ctrl p<0.05). Besides, increased leptin and adiponectin plasma level associated with expanded subcutaneous adipose depot and higher body weight were found in olz female offspring. Strikingly, for F2 olz-offspring, both male and female mice devel-

oped a marked glucose intolerance, hyperinsulinemia and insulinoreistance compared to F2 controls, with a female-specific increase body weight (olz 4 vs ctrl p<0.05, olz 8 vs ctrl p<0.01).

Conclusion: These data suggest that in utero antipsychotic exposure is sufficient to provoke sharp health outcomes in sex-specific manner, indicating varying developmental vulnerabilities between sexes towards metabolic diseases. They also evidence the transgenerational programming of diabetes susceptibility in response to maternal antipsychotic treatment.

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PS 051 The role of the liver in glucose and lipid metabolism

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Fat accumulates preferentially in the right liver lobe in non-diabetic subjects: implications for the diagnosis of NAFLD

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Background and aims: The right lobe of the liver preferentially receives blood flow from superior mesenteric vein, while the left lobe mainly drains the spleen. In obese subjects, the rate of visceral lipolysis is increased compared to non-obese subjects and can account for up to 50% of free fatty acids (FFA) delivery to the liver. Many obese subjects accumulate fat in the liver due to non-alcoholic causes (NAFLD) and therefore possibly an increased flux of FFA from visceral fat to the superior mesenteric vein. Studies using Doppler ultrasonography in healthy volunteers have shown that in response to a meal, intrahepatic portal vein blood flow increases more in the right than the left lobe. FFA delivery from both the meal and from visceral lipolysis might preferentially increase liver fat content more in the right than the left lobe. Metabolic consequences of hepatic insulin resistance in NAFLD such as hyperinsulinemia and hypertriglyceridemia could therefore also be better correlated with fat in the right than the left lobe. We aim to examine the distribution of liver fat (LFAT) in non-diabetic subjects and to test whether the fat in the right as compared to the left lobe correlate better with components of the metabolic syndrome or not.

Materials and methods: We determined LFAT by ¹H-MRS in the right lobe (LFAT%MRS), and by MRI (LFAT%MRI) in four regions of interest (ROIs 1–4, ROI1–2 in the left and ROI 3–4 in the right lobe) in 97 carefully phenotyped non-diabetic subjects (age range 22–74 yrs, BMI 18–41 kg/m²) and compared the accuracy of LFATMRI in the different ROIs in diagnosing non-alcoholic fatty liver disease (NAFLD) using areas under the receiver operator characteristic (AUROC) curves.

Results: 38% of the subjects had NAFLD (LFAT%MRS). LFAT%MRI in ROIs 1, 2, 3 and 4 averaged: 4.8±0.5%, 5.5±0.5%, 5.8±0.5% and 5.7±0.5%. The LFAT%MRI was significantly lower in ROI 1 than in ROI 2, ROI 3 and ROI 4. LFAT%MRI was significantly higher in the right (5.7±0.5%) than the left (5.1±0.4%) lobe ($p<0.02$). LFAT%MRI in ROI 3 showed excellent accuracy in diagnosing NAFLD (AUROC = 0.936±0.023, $p<0.001$, sensitivity 94.6%, specificity 83.3%). The AUROC for ROI 1 was good (AUROC = 0.879±0.034, $p<0.001$, sensitivity 70.3%, specificity 90.0%) but significantly lower than that of AUROC for ROI 3 ($p<0.02$). Likewise, the AUROC was significantly higher using LFAT%MRI in the right (AUROC = 0.934±0.025, $p<0.001$, sensitivity 86.5%, specificity 90.0%) than in the left lobe (AUROC = 0.894±0.032, $p<0.001$, sensitivity 73.0%, specificity 91.7%) lobe ($p<0.05$). The correlation coefficients between BMI, waist and hip circumference, body fat, intra-abdominal and subcutaneous fat, S-AST, S-ALT and LFAT%MRS were significantly higher than those between these metabolic features and LFAT%MRI in ROI 1. The correlation coefficient between HOMA-IR, S-AST, S-ALT and LFAT% MRI in ROI 2 was significantly lower than those between LFAT%MRS and these metabolic features. The correlation coefficients between the metabolic parameters and LFAT%MRI in ROIs 3 and 4 did not differ from those relating the respective parameters and LFAT%MRS.

Conclusion: Measurement of fat in the right lobe provides a more accurate diagnosis of NAFLD than fat in the left lobe.

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Pivotal role of TNF-alpha in the development and progression of non-alcoholic fatty liver disease in a murine model

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) represents the hepatic component of the metabolic syndrome, incorporating a wide spectrum of liver disease from simple steatosis to non-alcoholic stea-

tohepatitis (NASH) leading to cirrhosis and ultimately hepatocellular carcinoma. However, mediators of this progression are incompletely understood. Previously we have reported that adipocyte-specific nSREBP-1c transgenic (Tg) mice spontaneously developed hepatic lesions similar to those of human NASH. nSREBP-1c Tg mice are insulin resistant and their circulating levels of TNF-alpha are significantly higher than wild-type mice. In this study we assessed the role of TNF-alpha in the progression of NAFLD.

Materials and methods: We established a Tnf(-/-) nSREBP-1c Tg mouse line on C57B/6 background. Liver sections were stained with hematoxylin and eosin or azocarmine and aniline blue (Azan). mRNA levels of acetyl-CoA carboxylase (Acc1), stearoyl-CoA desaturase-1 (Scd1), monocyte chemoattractant protein-1 (Mcp1), transforming growth factor-beta (Tgfb1), collagen type I alpha 1 (Col1a1), and tissue inhibitor of metalloproteinase 1 (Timp1) were determined by quantitative reverse transcription PCR. Timp1 protein levels were assessed by Western blotting and immunohistochemical staining. Primary hepatocytes isolated from Tnf(-/-) C57BL/6 mice were cultured in Williams' medium E.

Results: All of 17 nSREBP-1c Tg mice had fatty liver at the age of 20 weeks. However, significant fat deposits were observed only in 5 of 20 age-matched Tnf(-/-) nSREBP-1c Tg mice ($p<0.0001$). When compared with nSREBP-1c Tg mice, the prevalence of liver fibrosis was low in Tnf(-/-) nSREBP-1c Tg mice (11/17 vs. 3/20, $p=0.006$). nSREBP-1c Tg mice showed higher expression of Acc1, Scd1, Mcp1, Tgfb1, Col1a1, and Timp1 in the liver than wild-type C57BL/6 mice. The protein levels of Timp1 were also increased in the liver from nSREBP-1c Tg mice. The expression levels of the genes and the Timp1 protein levels were reduced in Tnf(-/-) nSREBP-1c Tg mice. Incubation with 10 ng/ml TNF-alpha up-regulated the Timp1 expression in primary cultured hepatocytes from Tnf(-/-) C57BL/6 mice.

Conclusion: These observations indicate that TNF-alpha is involved in the development of NAFLD through the induction of key enzymes of lipid metabolism, ACC1 and SCD1. TNF-alpha was also associated with the increase of inflammatory cytokines, MCP1 and TGF-beta. Several studies have shown that TIMP1 is increased in liver fibrosis both in murine models and human samples, and promotes liver fibrosis development. Here we showed that Timp1 mRNA was decreased in the liver from Tnf(-/-) mice, and the supplementation of TNF-alpha increased the expression of Timp1 in primary cultured Tnf(-/-) hepatocytes. Thus TNF-alpha may play a pivotal role in both the development of NAFLD and progression to NASH in insulin resistant state.

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Proteomic analysis of livers from fat-fed mice deficient in either PKCδ or PKCε

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Background and aims: The two isoforms of protein kinase C (PKC), PKCδ and PKCε, have been associated with insulin resistance in the liver, which is a characteristic of Type 2 diabetes. Our previous comparison between PKCδ knockout (KO) mice and PKCε KO mice revealed roles for both of the kinases in the generation of insulin resistance, yet opposing roles in hepatic triglyceride accumulation. Therefore we aim to gain mechanistic insights into the roles of PKCs in the modulation of insulin action and lipid metabolism using a proteomics approach.

Materials and methods: We conducted an unbiased *in vivo* spike-in SILAC proteomic profiling of livers from one week high fat diet-fed PKC KO mice and their wild-type counterparts.

Results: A total of 3359 proteins and 3488 proteins were identified from the PKCδ KO and PKCε KO study groups respectively, and we showed that several enzymes of lipid metabolism were affected by the fat diet. In fat-fed mice, 23 proteins showed significant changes upon PKCδ deletion while 19 proteins were affected by PKCε deletion. Notably, retinol pathway was significantly upregulated in fat-fed mice with either deletion. The majority of the proteins affected in this pathway belonged to the Cyp450 family which is involved in the metabolism of all-trans retinoic acid (atRA). Thus it is possible that alterations in atRA or its derivatives protect PKC KO mice from fat diet-induced insulin resistance. Furthermore, gene ontology (GO) analysis showed proteins involved in biological processes such as lipid biosynthesis and oxidation-reduction were enriched in the absence of either PKC isoform. However certain GO terms such as monosaccharide metabolism were enriched solely in PKCδ KO and

isoprenoid biosynthesis only in PKC ϵ KO. Taken together, these further suggest that PKC δ and PKC ϵ exert partly overlapping and differential effects on liver metabolism in response to fat oversupply.

Conclusion: These studies therefore provide a detailed comparison of the effects of fat feeding and deletion of PKC isoforms at the protein level, which will aid further understanding of the function of these PKCs, given their association with defective glucose homeostasis.

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The hepatokine betatrophin is increased in nonalcoholic fatty liver disease and may affect insulin secretion in prediabetes

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Background and aims: Animal data suggest that the liver-secreted protein betatrophin is elevated in insulin resistant states and improves glucose tolerance by expanding beta cell mass. We now investigated whether betatrophin is a hepatokine that may be increasingly produced in nonalcoholic fatty liver disease (NAFLD) and may affect insulin secretion in humans.

Materials and methods: In liver tissue samples from 92 donors the relationship of hepatic betatrophin mRNA expression with liver triglyceride (TG) content, and in a subgroup of 29 donors the relationship of betatrophin mRNA expression with circulating betatrophin was studied. The associations of the cholesterol-regulating single nucleotide polymorphism (SNP) rs2278426 in the betatrophin-encoding gene DOCK6, with insulin secretion was studied in a cross-sectional study (N=2136) and during 9 month of a lifestyle intervention (N=344).

Results: Betatrophin mRNA expression correlated positively with liver TG content ($r=0.31$, $p=0.0025$). In addition, a positive relationship was found between betatrophin mRNA expression and betatrophin plasma levels ($r=0.41$, $p=0.03$). The minor T allele of the SNP rs2278426, that also associated with lower plasma cholesterol levels in the present study ($p=0.002$), associated with higher insulin secretion, adjusted for sex, age and insulin sensitivity, in subjects with prediabetes (N=701, $p=0.044$), but not in subjects with normal glucose regulation (N=1435, $p=0.78$). The minor T allele of the SNP rs2278426 also predicted a larger increase in adjusted insulin secretion during the lifestyle intervention in subjects with prediabetes ($p=0.01$) and associated with higher arginine-stimulated insulin secretion ($p=0.04$) in 97 subjects during a hyperglycaemic clamp.

Conclusion: We provide novel information that the liver-secreted protein betatrophin is increasingly expressed in NAFLD and that it may not only be involved in cholesterol metabolism, but also in glucose metabolism in subjects who are at increased risk for type 2 diabetes.

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Acute elevation of triglycerides and free fatty acids does not modulate plasma betatrophin levels in healthy individuals

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Background and aims: Betatrophin (ANGPTL8) is a liver-derived protein, and its overexpression in liver has been shown to cause beta cell proliferation, increased insulin secretion and hypertriglyceridemia in mice. ANGPTL8 knockout leads to marked reduction in postprandial triglyceride levels in mice. However, the relationships between plasma triglycerides, free fatty acids (FFA), and betatrophin have not been studied in humans.

Materials and methods: We examined the effect of acute elevation of plasma triglycerides and FFA on plasma betatrophin levels in 9 healthy NGT subjects (7 male/2 female, age = 42 ± 4 yrs, BMI = 26.3 ± 1.0 kg/m², FPG = 90 ± 3 mg/dl, 2h PG = 135 ± 11 mg/dl), without family history of T2DM. Subjects received on two different occasions either a lipid (intralipid 60 ml/h) or saline

(0.9% NaCl 60 ml/h) infusion for 6 hours followed by euglycemic insulin clamp (80 mU/m².min) for 2 hours. Plasma triglyceride, FFA, and betatrophin levels were measured at baseline, 6 hours, and 8 hours.

Results: Plasma triglyceride levels during the lipid infusion increased from 187 ± 39 mg/dl at baseline to 455 ± 59 mg/dl at 6 hours and declined to 398 ± 62 mg/dl at 8 hours, and during the saline infusion decreased from 183 ± 60 mg/dl to 129 ± 28 mg/dl at 6 hours and 106 ± 25 mg/dl at 8 hours. Plasma FFA levels during the lipid infusion increased from 370 ± 39 μ M to 846 ± 68 μ M at 6 hours and declined to 496 ± 66 μ M at 8 hours. Plasma FFA concentrations did not change during the saline infusion. Insulin-mediated glucose disposal (Rd) was reduced during the lipid versus saline infusion (9.08 ± 1.5 vs 11.3 ± 1.4 mg/kgFFM.min, $p = 0.02$). Plasma betatrophin concentrations during the lipid infusion were 22 ± 15 , 27 ± 16 , and 27 ± 17 ng/ml at 0, 6, and 8 hours, respectively ($p=ns$). The corresponding values of betatrophin during the saline infusion were 21 ± 6 , 18 ± 6 , and 24 ± 5 ng/ml, respectively. There was no relationship between plasma triglyceride, FFA, betatrophin concentrations, or insulin mediated whole body glucose disposal.

Conclusion: Short-term physiologic increase in plasma FFA, and triglycerides induce insulin resistance in skeletal muscle, but does not affect plasma betatrophin levels in healthy subjects. The association between betatrophin and lipids reported in animals is not observed in humans.

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ZBTB20 regulates lipid metabolism

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Background and aims: We aims to investigate the potential role of novel zinc finger protein ZBTB20.

Materials and methods: We established liver-specific Zbtb20 knockout mice (LZB20KO) by Cre/LoxP, analyzed phenotypes, determined gene expression by RT-PCR and Western blot, and examined protein/DNA interaction by ChIP and reporter assay.

Results: Specific deletion of ZBTB20 in the liver resulted in a marked reduction in plasma lipid levels and hepatic triglyceride contents, and a significant improvement of high carbohydrate diet-induced hepatic steatosis and insulin resistance. Hepatic lipogenesis was substantially impaired in the absence of ZBTB20, which was associated with decreased expression of ChREBP- α and its target genes critical for glycolysis and lipogenesis, and could in part be restored by overexpression of ChREBP- α . Furthermore, liver ZBTB20 expression was significantly increased in ob/ob mice. Strikingly, liver-specific deletion of ZBTB20 in ob/ob mice could dramatically suppress hepatic lipogenesis, and improve obesity-induced hyperglycemia, dyslipidemia, hepatic steatosis, and insulin resistance.

Conclusion: Together, these data indicate that liver ZBTB20 is essential for lipid metabolism and may serve as a therapeutic target for metabolic syndrome.

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The role of starvation-induced autophagy in gluconeogenesis and ketogenesis

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Background and aims: During starvation, fatty acids released from adipose tissue are mainly used for hepatic gluconeogenesis and ketogenesis to maintain systemic energy homeostasis in animals. Autophagy, a major intracellular degradation system, is induced by starvation. Systemic autophagy-deficient mice die in neonatal starvation period, suggesting that autophagy is a critical survival response against starvation. However, the exact role of starvation-induced autophagy in systemic energy homeostasis remains unclear.

Materials and methods: To determine the importance of autophagy in each organ, we examined starvation-induced gluconeogenesis and ketogenesis in liver specific Atg5 knockout mice (L-Atg5^{-/-}), kidney proximal tubular epithelial cells specific Atg5 knockout mice (K-Atg5^{-/-}), and their control mice (Atg5lox/lox).

Results: Under ad-libitum feeding condition, there were no significant differences in plasma ketone levels. But under 36-h fasting condition, increase in plasma ketone levels was impaired in L-Atg5^{-/-} mice but not in K-Atg5^{-/-} mice. The impairment of ketogenesis in L-Atg5^{-/-} mice was incomplete. In

addition, the oil-red-O-stained lipid accumulation as a main source of ketogenesis and elevation of expression of HMG-CoA synthase, a rate-limiting enzyme of ketogenesis were observed in the kidney likely in the liver. We then hypothesized that the kidney compensated for the absence of ketone in L-Atg5^{-/-} mice. To examine this hypothesis, we generated the mice lacking Atg5 in both the liver and kidney. These mice showed further decline in plasma ketone concentrations compared to L-Atg5^{-/-} mice during a 36-h fast. Starvation-induced lipid droplets formation was impaired in Atg5-deficient organs. No significant differences in blood glucose levels and the expression of some enzymes for gluconeogenesis were observed among each type of mice, even with starvation.

Conclusion: These results suggest that starvation-induced autophagy is essential for ketogenesis relating with lipid accumulation from free fatty acid, and that the kidney potentially has a function to generate ketone bodies.

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PS 052 Mitochondrial function, oxidative stress and glucose metabolism

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The Cohen diabetic rat: a matter of mitochondrial disorder?

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Background and aims: The Cohen diabetic rat model consists of two strains: the Cohen diabetic sensitive rat (CDs) developing hyperglycemia and decreased glucose stimulated insulin secretion (GSIS) on the diabetogenic-high-sucrose diet (HSD) and the Cohen diabetic resistant rat (CDr) which remains normoglycemic on HSD. Although underlying mechanisms causing inhibited GSIS in CDs remains yet elusive, we recently showed reduced activity of the mitochondrial respiratory-chain enzyme, cytochrome *c* oxidase (COX) and ATP content in islets of hyperglycemic-CDs, tightly linking inhibited GSIS to COX deficiency. We examined whether CDs has a ubiquitous COX deficiency, thus suggesting a model of mitochondrial disorder related diabetes.

Materials and methods: CDs and CDr males, eight weeks old were fed a regular-diet (RD) or a diabetogenic-diet containing 72% sucrose for 30 days. Activity of COX normalized to citrate-synthase (CS) was determined in pancreatic-islets, lymphocytes, pancreas, heart and liver homogenates spectrophotometrically. Expression levels of COX subunits 1 & 2 (COX1 & COX2) and their chaperons Sco1, COX11 and COX17 were examined in RNA extracted from islets by qRT-PCR and expressed as a fold-change of the expression in CDr fed RD. Mitochondrial DNA (mtDNA) content was analyzed in DNA from islets by qRT-PCR. COX1 protein levels were determined in islets by Western-blot analysis.

Results: An initial significant decrease in COX activity was observed in islets, lymphocytes, pancreas, heart and liver of normoglycemic-CDs demonstrating 85, 40, 30, 40 and 30% respective reduction ($p < 0.01$) compared to CDr fed RD. On the diabetogenic-HSD, COX activity was significantly ($p < 0.01$) reduced in hyperglycemic-CDs and normoglycemic-CDr but the decrease in hyperglycemic-CDs was more evident. Activity of the ubiquitous mitochondrial matrix enzyme CS used as an estimation of mitochondrial content was not different between groups. Islets COX1 and COX2 expression levels were 0.3-fold reduced in CDs fed RD, ($p < 0.01$) and Sco1, COX11 and COX17, COX assembly proteins, were 0.5, 0.3 and 0.2 fold reduced respectively ($p < 0.01$), compared to CDr. COX1 protein levels were also reduced in islets of CDs fed RD. mtDNA content was 0.5-fold decreased ($p < 0.01$) in islets of CDs fed RD, compared to CDr.

Conclusion: We demonstrate a widespread impairment of COX activity in islets, lymphocytes, pancreas, heart and liver of the normoglycemic-CDs deteriorating furthermore on diabetogenic diet. The more prominent COX deficiency observed in islets presumably explains why the clinical outcome in CDs is mainly manifested as diabetes, while the phenotype is milder in the other tissues. Moreover, alterations in mitochondrial function are likely to have deleterious effects in cells that have a high energy requirement, such as the β -cell. These finding links COX deficiency and β -cell dysfunction and positions the Cohen diabetic rat as a good model to study mitochondrial disease-related diabetes.

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Weight loss in obese ob/ob mice improves mitochondrial dynamics in liver, muscle and adipose tissue

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Background and aims: Mitochondrial dysfunction has been proposed to play an important role in the development of type 2 diabetes. Six proteins are currently known in mammals regulating the mitochondrial live cycle. The Mitofusin 1 and 2 (Mfn1/2) and the optic atrophy protein 1 (Opa1) are essential for mitochondrial fusion, whereas the fission protein 1 (Fis1), the

mitochondrial fission factor (Mff) and the dynamin related protein 1 (Drp1) control fission. Recent studies indicate that obesity deteriorates mitochondrial dynamics, and thus, mitochondrial function. In this study we investigated regulation of mitochondrial dynamics in liver, muscle and adipose tissue of ob/ob mice after calorie-restricted feeding compared to ad-libitum feeding.

Materials and methods: Three month old male obese ob/ob (B6.V-lebob/ob) mice were fed for twelve weeks with a calorie-restricted diet, whereas the control group had unrestricted access to feed. Age matched ob/+ (B6.V-lebob/+) mice served as lean controls. Finally mice were killed and tissues were analyzed for Mfn1/2, Opa1, Fis1, Mff and Drp1 expression by quantitative PCR analyses. Fis1 protein expression was investigated using western blot and immunofluorescence analyses. The mitochondrial network was visualized by Mito Tracker DeepRed staining and fat accumulation by Red Oil staining.

Results: At the age of three month ob/ob mice showed a significantly higher body weight than ob/+ mice (46 ± 2 vs. 31 ± 1 g). After twelve weeks of calorie-restricted diet obese ob/ob mice showed a significantly lower body weight compared to ad-libitum feed mice (39 ± 2 vs. 62 ± 2 g). In addition a lower level of fat accumulation in liver and muscle was observed in ob/ob mice after calorie-restricted diet. Gene expression of all fusion and fission proteins was lower in liver, muscle and adipose tissue of ob/ob mice compared to lean control mice, indicating reduction of mitochondrial dynamics. Calorie-restricted diet resulted in ob/ob mice in a significantly higher expression of all genes involved in mitochondrial fission and fusion compared to ad-libitum feeding. The strong increase of Fis1 expression in calorie-restricted feed ob/ob mice could be confirmed in liver, muscle and adipose tissue on the protein level. Obese ob/ob mice showed formation of mitochondrial cluster in liver and fatty tissue whereas a more homogenous network of mitochondria was observed in diet feed ob/ob mice.

Conclusion: We observed a correlation between reduced body weight and lower fat accumulation and improved mitochondrial dynamics in liver, muscle and adipose tissue of ob/ob mice. Thus, a calorie-restricted diet has a beneficial effect on mitochondrial morphology in obese subjects. Mitochondrial function in liver, muscle and adipose tissue is significant to maintain insulin sensitivity and glucose tolerance. Our study provides further evidence that obesity induced mitochondrial dysfunction contributes to the development of type 2 diabetes.

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An mtDNA mutation in the cytochrome c oxidase changes mitochondrial dynamics and favours development of type 2 diabetes mellitus

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Background and aims: Mutations in the mitochondrial genome (mtDNA) affect subunits of respiratory chain complexes and can impair their function. It has been postulated that mtDNA mutations which accumulate with age contribute to the pathogenesis of type 2 diabetes mellitus (T2DM). We have shown that the conplastic mouse strain C57BL/6NTac-mtNOD/AtJ (mtNOD) with a mtDNA point mutation in the subunit 3 of cytochrome c oxidase (OXPHOS complex IV) has a higher ROS production with age compared to controls. In this study we investigated mitochondrial morphology and mitochondrial fission-fusion processes in hepatocytes of this conplastic mouse strain.

Materials and methods: Primary hepatocytes were isolated from 3, 6, 9 and 12 month old mtNOD and C57BL/6NTac (control) mice. Hepatocytes were stained with MitoTrackerGreen. Thereafter mitochondrial morphology was investigated by fluorescence microscopy and the median mitochondrial elongation level was calculated using AutoQuant software. Gene expression of the mitochondrial fusion proteins OPA1, MFN1 and MFN2 and of the fission proteins FIS1 and DNMI1 was determined by quantitative PCR analyses.

Results: The median mitochondrial elongation level in 3 month old hepatocytes of mtNOD mice was significantly lower compared to control mice. The median mitochondrial elongation level decreased with age in hepatocytes of control mice. In contrast, an increase of the median mitochondrial elongation level was observed in hepatocytes of mtNOD mice. Gene expression of the mitofusin MFN1 and MFN2 declined during ageing in control mice, whereas the expression of both genes increased in mtNOD mice. At the age of 12 month the expression was in mtNOD mice twice as high as in control mice. In addition, the expression of the fission protein DNMI1 was significantly lower in 12 months old mtNOD compared to control mice.

Conclusion: The observed mitochondrial phenotype correlated with the gene expression profile of mitochondrial fission and fusion genes at the

investigated points of age. Our results indicate that a mtDNA mutation in the cytochrome c oxidase triggers a higher mitochondrial elongation level of hepatocytes with age. This process could be mediated by an interaction between the higher ROS production and the up-regulation of MFN1 and MFN2 in aged mtNOD mice. Thus, the C57BL/6NTac-mtNOD/AtJ mouse strain represents an interesting model to study trigger effects of age dependent mitochondrial phenotypes in the pathogenesis of T2DM.

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Dual effect of Nd2 gene mutation in complex I of the respiratory chain: ROS generation and induction of mitoprotective effects in the course of ageing

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Background and aims: The mitochondrial genome of the diabetes-resistant B6-mtALR mouse strain is characterized by a mt-DNA encoded Nd2 polymorphism in complex I of the respiratory chain. In metabolically active organs such as the liver disorders of the mitochondrial electron transfer with increased ROS generation are linked to accelerated aging. It was the aim of the study to investigate the production of reactive oxygen species (ROS), the expression of antioxidant enzymes and the expression of the aging marker p53 in liver tissue of conplastic B6-mtALR mice compared to B6-mtAKR control strain.

Materials and methods: Conplastic B6-mtAKR (AKR) and B6-mtALR (ALR) mice were examined over a period of 2 years. AKR and ALR animals were killed and liver tissue was collected at the age of 3, 6, 9, 12, 18 and 24 months. The gene expression of Nd2, Sod1, Sod2, catalase, UCP2, TFAM and mTOR was quantified by real-time RT-PCR and the p53 protein expression was quantified by Western blot analyses. The measurement of mitochondrial ROS production in the liver was performed by fluorescence microscopy after injection of the ROS sensor Mitosox.

Results: The gene expression of mt-Nd2 in the ALR strain showed no differences compared to the control strain AKR. The ROS production in the liver at the ages of 6 and 12 months was significantly increased in AKR mice (2–18-fold, $p < 0.05$). Only at the age of 18 months ROS production in the liver of ALR animals was significantly increased by 100% ($p < 0.05$). Furthermore, the AKR strain showed higher expression of cytoprotective genes SOD2, SOD1 and catalase with advanced aging whereas for the ALR strain only at the time of impaired ROS generation an increased expression of UCP2 (3x, $p < 0.05$) and catalase (2.7x, $p < 0.05$) was detectable. The aging markers p53 showed a higher expression in the liver of AKR mice compared to the ALR strain with the mt-ND2 mutation (x3.5, $P < 0.01$).

Conclusion: The mt-ND2 mutation in complex I of the respiratory chain favors the formation of reactive oxygen species in the liver. The potential of increased ROS generation does not accelerate the aging process if antioxidant and mitoprotective genes provide sufficient protection as a process of mitohormesis. Only at advanced age the decreased expression of mitochondrial protective structures cannot counteract negative ROS effects on organ function and aging.

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The role of p66Shc in the development of obesity and metabolic disorders: a translational study

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Background and aims: p66Shc is a redox enzyme and adaptor protein, potentially associated with lifespan determination. Multiple evidences indicate that p66shc plays a role in the cellular response triggered by oxidative stress and the development of chronic diabetic complications. There are preliminary and contradictory data on the role of p66Shc in the development of obesity and type 2 diabetes. In this translational study, the role of p66Shc was evaluated in obesity and metabolic syndrome, studying the expression

of p66Shc in human adipose tissue and the characteristics of visceral adipose (VAT) and subcutaneous adipose tissue (SAT) in p66Shc-/-Ob/Ob mice at 18 and 30 weeks of age.

Materials and methods: 4 different mouse models were used: C57/BL6 wt mice, Ob/Ob mice, p66 -/- mice, p66 -/- Ob/Ob mice. 2 time points: 18 and 30 weeks of age. Both visceral (VAT) and sub-cutaneous (SAT) white adipose tissue samples were analysed.

Results: p66Shc-/-Ob/Ob mice develop obesity, but at 30 weeks, their body weight was significantly lower than in Ob/Ob controls. The size of visceral adipocytes was reduced in p66Shc-/- mice at 18 weeks, but not significantly different compared to control Ob/Ob mice at 30 weeks. The number of apoptotic cells in VAT was increased in Ob/Ob mice compared to wt, but significantly reduced by p66Shc deletion. The number of macrophages in VAT was also reduced in p66Shc-/-Ob/Ob compared to Ob/Ob mice. Based on ITT and GTT, p66Shc-/-Ob/Ob mice at 18 weeks are less glucose intolerant and insulin resistant compared to Ob/Ob mice, but become insulin resistant at 30 weeks of age. In a sample of 46 individuals undergoing bariatric surgery, the expression of p66Shc linearly correlates with BMI in VAT, but inversely correlates in SAT, only in young subjects.

Conclusion: These data support the hypothesis that p66Shc modulates the processes occurring during the development of obesity and metabolic disorders, affecting adipogenesis and inflammation of visceral adipose tissue. The role of p66Shc appears to be influenced by age, most likely in relation to the role of this protein during aging.

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Regulation of skeletal muscle lipolysis and oxidative metabolism by G0/G1 switch gene 2

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Background and aims: Intramyocellular triacylglycerol (IMTG) pool is an important energy source in skeletal muscle, especially during exercise. Recent data suggest that the *adipose triglyceride lipase* (ATGL) plays a key role in providing energy substrate from IMTG and perturbations of its expression/activity are linked to altered energy metabolism in skeletal muscle. However, little is known about its regulation. The aim of this study was to investigate the role of the protein *G0/G1 Switch Gene 2* (G0S2), recently described as an inhibitor of ATGL in white adipose tissue, in the regulation of lipolysis and oxidative metabolism in skeletal muscle.

Materials and methods: G0S2 was overexpressed and knocked down in human primary myotubes obtained from *rectus abdominis* biopsies in healthy volunteers and transduced with adenoviruses and lentiviruses, respectively. ATGL activity, IMTG content, fatty acid mobilization and oxidation, glucose oxidation and glycogen synthesis were assessed by using radiolabeled substrates. Metabolic studies were supplemented with the investigation of mitochondrial phenotype using fluorescent probes and RT-qPCR (for the analysis of key gene expression).

Results: Our results show that G0S2 is expressed in human myotubes and inhibits ATGL activity (-47% ; $P<0.05$). G0S2 downregulation induces a decrease in IMTG pool (-70% ; $P<0.001$) and an increase in fatty acid mobilization (+500% ; $P<0.001$) and oxidation (+300% ; $P<0.001$). Glucose oxidation and glycogen synthesis are decreased (-35% and -63% respectively ; $P<0.01$). The increase of fatty acid flux goes along with an increase in mitochondrial mass (+67% ; $P<0.001$) and membrane potential (+108% ; $P<0.01$). These effects may be mediated by the activation of *Peroxisome Proliferator Activated Receptor δ* (PPAR δ) target genes *Perilipin 2*, *PPAR Gamma Coactivator 1 α* and *Pyruvate Dehydrogenase Kinase 4* (+89%, +93%, and +295%, respectively ; $P<0.01$). Opposite effects are observed when G0S2 is overexpressed.

Conclusion: Collectively, the data indicate that G0S2 plays an important role in the regulation of human skeletal muscle lipolysis and oxidative metabolism, by modulating fatty acid flux and expression of PPAR δ target genes.

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Role of L-Carnitine on skeletal muscle hypertrophy and mitochondrial function

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Background and aims: Type 2 diabetes mellitus (T2DM) is a metabolic disease associated with hyperglycaemic condition due to impairments in insulin release and/or function. Insulin Resistance (IR) is defined as a reduced response to insulin biological effects in target tissues, like skeletal muscle. There is evidence that mitochondrial dysfunction is associated with IR and T2DM. In particular, oxidative stress has an important role in the inhibition of muscle metabolism: the progressive reduction in the number and efficiency of mitochondria has been proposed as a mechanism able to induce muscle atrophy in T2DM. L-Carnitine (CARN) is an essential nutrient and plays an important role in mitochondrial β -oxidation and in the ubiquitin-proteasome system regulation. Muscle cells are unable to synthesize CARN. As a dietary supplement to ameliorate pathological condition characterized by myofibrils degeneration or improve athletic performance, CARN has been studied for its potential to enhance β -oxidation. However, CARN effects on myogenesis, mitochondrial capacity and atrophy process are not completely elucidated. This study aims to investigate CARN action on skeletal muscle remodeling, in particular in differentiation and atrophy processes, in normal and insulin resistance conditions.

Materials and methods: We analyzed muscle cell differentiation and morphological features in C2C12 myoblasts exposed to 5 mM CARN. To study the RAN dose-response relationship we treated neo myotubes with 1, 5 and 25 mM CARN for 24 hours (h). 5 mM CARN turned out to be the dose able to stimulate morphological changes and hypertrophic process in neo-formed myotubes. 5 mM CARN was added to C2C12 during skeletal muscle proliferation, differentiation and on neo formed myotubes assessing cellular insulin response, mitochondrial function, oxidative stress pathways and atrophy genesis.

Results: Our data showed that CARN positively regulate the kinetics of C2C12 cell growth curve, accelerate myotubes formation and induce morphological changes indicating the start of hypertrophy process. In addition, CARN improved AKT phosphorylation and downstream cellular synthetic pathways. CARN positively regulated mitochondrial biogenesis whilst negatively modulated the pathways related to oxidative stress/ROS productions (SOD modulation).

Conclusion: We provide an interesting novel mechanism of the potential therapeutic use of CARN to treat insulin resistance condition characterized by skeletal muscle morphological and functional impairment, mitochondrial dysfunction and oxidative stress production in atrophy process.

PS 053 Metabolic effects of dietary composition, caloric restriction and bariatric surgery

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Associations of dietary fibre intake and risk factors of the metabolic syndrome. The lipid and glucose under prospective surveillance (LUPS) study

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Background and aims: The metabolic syndrome is classified by a clustering of metabolic and cardiovascular risk factors, in particular, elevated waist circumference, serum triglycerides, blood pressure, fasting glucose and reduced HDL-cholesterol. The exact etiology remains unclear, but it is known to be a complex interaction between genetic, metabolic, and environmental factors. Amongst exogenous factors nutrition has a central role in the development of the metabolic syndrome, and dietary fibre intake is supposed to be an important factor in this respect. Therefore we aimed to evaluate associations of dietary fibre intake and the prevalence of risk factors of the metabolic syndrome in the LUPS cohort.

Materials and methods: Baseline data from the prospective LUPS cohort (1887 healthy workers, 1274 men and 613 women, aged 25–60 years) were investigated for associations between dietary fibre intake and risk factors of the metabolic syndrome (classification criteria) using logistic regression and Jonckheere-Terpstra test. Fibre intake was assessed by a standardized semi-quantitative food frequency questionnaire with 85 items covering typical food choices and portion sizes in Germany.

Results: Mean dietary fibre intake (g/1000 Kcal/day) was 11.9 (95% CI: 11.6; 12.3) in women and 9.9 (95% CI: 9.7; 10.1) men. The highest prevalence of risk factors for the metabolic syndrome was seen for increased blood pressure in 70% of men and 40% of women. The prevalence of the other risk factors of the metabolic syndrome in the cohort decreased from elevated waist circumference (28%), high triglycerides (20%), reduced HDL-cholesterol (19%) to increased fasting blood glucose (10%). Only elevated waist circumference showed a higher prevalence in women (37%) than in men (25%). Significant negative associations were found between fibre intake and waist circumference (−6.36 standardized JT-statistics with continuous variable, odds ratio 0.952 for grouped variable; waist circumference for men ≥ 102 cm and ≥ 88 cm for women) as well as for fibre intake and fasting triglyceride levels (−5.57 standardized JT-statistics, odds ratio 0.927; triglycerides ≥ 150 mg/dl), while positive associations were seen between fibre intake and HDL-cholesterol (8.66 standardized JT-statistics, odds ratio 0.948; HDL-cholesterol < 40 mg/dl for men and < 50 mg/dl for women) (all $p < 0.05$), however no associations were observed between fibre intake, and elevated blood pressure ($\geq 130/85$ mmHg) as well as fibre intake and increased fasting glucose (≥ 100 mg/dl) in the baseline investigation of the LUPS cohort.

Conclusion: Dietary fibre intake, in the range commonly consumed by healthy workers, was significantly related to important risk factors of the metabolic syndrome in the LUPS study. Higher fibre intake was associated with smaller waist, lower triglycerides and higher HDL-cholesterol.

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Dairy lipids, proteins, and abdominal obesity (DairyHealth): a 12-week, randomised, parallel-controlled, human intervention study

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Background and aims: Abdominal obesity is associated with increased risk of cardiovascular disease (CVD), type 2 diabetes, and overall mortality. Obesity leads to exaggerated postprandial lipaemia (PPL), an independent risk factor for CVD. Food quality has been found to affect PPL. Milk protein, especially whey and milk fat naturally enriched with short- and medium-

chain saturated fatty acids (SMC-SFA) seem to possess health beneficial effects. However, long-term human intervention studies are lacking. The aim was to test if whey protein and milk fat enriched in SMC-SFA has beneficial and synergistic effects on the postprandial lipid responses.

Materials and methods: 12-week, randomized, double-blinded, human intervention study. 63 subjects were randomized to one of four diets in a 2x2 Latin square design. Participants consumed daily 60g milk protein (whey or casein) and 63g milk fat (two different SMC-SFA compositions) daily. Before and after the 12-week intervention a high-fat meal test (65 E% fat, 19 E% carbohydrates, and 16 E% protein; 4,500 kJ) was performed and changes from baseline in postprandial triglyceride, ApoB-48 (reflecting chylomicrons of intestinal origin), free fatty acids (FFA), insulin, glucose and glucagon were measured. Two-way ANOVA analysis was used. Power calculation showed that we needed 13 in each group to detect a difference of 20% in triglyceride response ($\alpha = 0.05$, $\beta = 0.80$).

Results: 52 subjects completed the study. Baseline characteristics were similar between the four groups. We found that the ApoB-48 response decreased significantly after whey compared to casein ($P = 0.025$). We found no significant differences in postprandial triglyceride, FFA, insulin, glucose or glucagon responses to whey versus casein or to the two different SMC-SFA compositions. No interaction between protein and fat was observed.

Conclusion: 12-week intervention with whey supplementation resulted in a decreased postprandial chylomicron response in subjects with abdominal obesity. This opens for the development of whey enriched dairy products that may possess beneficial effects on the risk of CVD.

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Lysophospholipids in human blood and their interaction with white adipose tissue under an isocaloric high-fat diet in the NUGAT-study

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Background and aims: Diets high in saturated fats increase diabetes risk and fatty liver. Apart from the cholesterol metabolism their detailed effects on the lipids are not well established which we therefore studied using cutting edge technology.

Materials and methods: In the NUGAT study (NUtriGenomic Analysis in Twins) we used a twin-design with 92 healthy participants and two isocaloric nutritional interventions to investigate the impact of a six-week carbohydrate rich, low fat followed by a six-week high fat low carbohydrate diet. Extensive characterization of the metabolic responses was performed after the low fat (LF) and after 1 (HF1) or 6 (HF6) weeks of high fat diet to evaluate rapid and long-term effects. Apart from other 145 lipids, 20 lysophospholipids were analyzed within and in connection with measured biomarkers of blood and white adipose tissue. The fold changes (FC) between two clinical investigation days were calculated and the Benjamini-Hochberg method (BH) was used to adjust the global significance level for multiple testing.

Results: Lysophosphatidylcholines (LPC) and -ethanolamines (LPE) showed an almost continuous decrease during the six weeks on high-fat diet (LPC: $FC_{LF, HF6} \approx 0.55$; LPE: $FC_{LF, HF6} \approx 0.73$), whereas other measured lipid classes reacted biphasically and more adaptively. Members of LPC as well as LPE were also highly connected within and between these two lipid classes, partly reflecting their known biosynthesis. The lysophospholipids were associated with biomarkers of signal transduction, apoptosis (i.e. SQSTM1/p62: $\tau \approx 0.36$, $p_{BH} < 0.05$) as well as members of inflammatory pathways and the immune system (i.e. TBX21: $\tau \approx -0.41$, $p_{BH} < 0.05$). These associations were not only given for basal values but also for differences between the clinical investigation days. The gene expression of NFKBIA/IkBa, which decreased significantly during the study ($FC_{LF, HF6} \approx 0.80$), also showed high associations with the LPEs and LPCs (i.e.: $\tau \approx 0.40$, $p_{BH} < 0.001$). However, TLR2 and TLR4 were independent of the lysophospholipids (TLR2: $p_{BH} > 0.41$; TLR4: $p_{BH} > 0.31$).

Conclusion: Although extensive adaptation occurs in our healthy participants after 6 weeks on a high-fat diet, parts of the metabolism remain disturbed. The decreasing lysophospholipids seem to accompany the activation of the immune system as well as higher inflammatory processes. Our analysis

shows novel pathways by which saturated fats increase inflammation independent of the known TLR4-related mechanisms of inflammation.

Clinical Trial Registration Number: NCT01631123

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The effect of meal frequency on fatty acid composition in serum phospholipids in patients with type 2 diabetes

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Background and aims: Fatty acids are important cellular constituents that may affect many metabolic processes relevant for the development of diabetes and its complications. We demonstrated previously the superior effect of two meals a day, breakfast and lunch (B2) on body weight, hepatic fat content and insulin sensitivity compared to the same diet divided into six smaller meals a day (A6). The aim of this secondary analysis was to explore the effect of frequency of meals on the fatty acid composition of serum phospholipids in subjects with type 2 diabetes (T2D).

Materials and methods: In a randomised, crossover study, we assigned 54 patients with T2D to follow two regimens of a hypocaloric diet (-500 kcal/day), each for 12 weeks: six meals (A6), and two meals a day, breakfast and lunch (B2). The diet in both regimens had the same macronutrient and energy content. The procedures were performed at weeks 0, 12 and 24. The fatty acid composition of serum phospholipids was measured by gas liquid chromatography. Insulin sensitivity was derived as an oral glucose insulin sensitivity (OGIS) index.

Results: Saturated fatty acids (mainly the myristic and palmitic acids) decreased ($p<0.001$) and n6 polyunsaturated fatty acids increased ($p<0.001$) in response to both regimens, more with B2 ($p<0.001$ for both). Monounsaturated fatty acids decreased ($p<0.05$) and n3 polyunsaturated fatty acids increased ($p<0.001$) in response to both regimens with no difference between the treatments. The increase in OGIS correlated positively with changes in proportion of linoleic acid in B2. This correlation remained significant even after adjustment for changes in BMI ($r=+0.38$; $p=0.012$).

Conclusion: We demonstrated that meal frequency affects fatty acid composition of serum phospholipids. We observed more marked positive effects of B2. The increase in linoleic acid could partly explain the insulin-sensitising effect of B2 in T2D.

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Early undernutrition worsens the metabolic effects of high-lipid diets

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Background and aims: Early undernutrition is associated with increased risk of diseases in adulthood. Interventions on nutrition of mothers and children have short-term benefits on health but growth-retarded offspring overfed undergoes a rapid catch-up growth and become long-term heavier than normal. Paradoxically, the West's obesity epidemic is spreading into developing countries in conjunction with a high prevalence of malnutrition. This raises the question whether previous undernutrition exacerbates the metabolic alterations associated with calorie-dense foods. Published results are contradictory about. The aim of present study is: A) to investigate the impact of undernutrition in hypothalamus; B) to explain how this condition worsens some alterations associated with subsequent high-fat diets.

Materials and methods: Wistar rats were subjected to undernutrition from 14th d of foetal life until 70 d (U). Control (C) animals were fed a standard chow ad libitum (3% fat). From 70 days (C70d, U70d) until 9 m, several groups were established: C70 was segregated into two groups, given the commercial chow (C9m) or a cafeteria diet (C9mCaf) of high lipid concentration, but lower than used in most similar studies (13% fat). A group of undernour-

ished rats was kept under restriction (U9m), other was transferred to cafeteria diet (U9mCaf) and other was transferred to commercial chow (U9mC). Adiposity was calculated by RMI. Hypothalamic POMC and NPY mRNA, as well as hepatic MCP-1 mRNA, were quantified. Insulin sensitivity was assessed by euglycemic clamp.

Results: Calorie intake of restricted rats under the high-fat diet exceeded that of controls, which was consistent with a reduced POMC protein and increased NPY expression. These alterations could derive from an impaired neonatal "leptin surge". All rats feeding cafeteria diet weighed more than their matched standard chow animals. In controls, this implied a mild obesity, not found in the pre-restricted. The relative increase in visceral fat over previous content was higher in U9mCaf than in C9mCaf rats: 6-fold vs. 2-fold, respectively. Insulin-resistance was established in both groups. Hyperlipidemia and triglyceride deposits were increased only in liver and muscle of U9mCaf rats, in which a marked increase of hepatic MCP-1 mRNA was also found.

Conclusion: Metabolic effects of a cafeteria diet are worsened by previous undernutrition, specifically: ectopic lipid storage and dislipemia. Also, increased MCP-1 in liver suggests accumulation of inflammatory macrophages, as hepatic injury in chronic liver diseases. These results indicate that previous food restriction increases vulnerability to the harmful effects of fatty diets.

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Severe food restriction induces stress and adipose tissue inflammation, contributing to the promotion of insulin resistance

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Background and aims: Accumulation of proinflammatory immune cells in adipose tissue contributes to the development of obesity-associated disorders. To reduce of obesity, food restriction has been recommended as an effective therapeutic strategy for weight loss. Many studies have shown that food-intake schedule can alter biological rhythms. Here, we studied whether food restriction program induces stress and cause immune cells infiltration in adipose tissue, contributing to the insulin resistance.

Materials and methods: C57BL/6 mice were fed 60% high fat diet for 8 weeks and randomly divided into three groups: the control group was continuously fed 60% high fat diet (100%); the 50% food restriction groups were fed 50% of the mean amount of food consumed by the control mice, fed 50% once a day (FR1; 50%-1, 9:00 AM) or fed 25% twice a day (FR2; 25%-2, 9:00 AM, 9:00 PM) for 3 days. Body weight was monitored and immune cell infiltration of epididymal adipose tissue was examined by hematoxylin and eosin staining. In addition, the expression of lipolysis or inflammatory related genes in adipose tissue was analyzed by qRT-PCR and the serum corticosterone levels were measured by ELISA. To investigate the association between stress hormone and adipose tissue inflammation, high fat diet induced obese mice were treated with vehicle or corticosterone (15 ug/h via an osmotic minipump, subcutaneously) for 3 days, and the expression of lipolysis and inflammatory related genes was analyzed by qRT-PCR, blood glucose levels were measured, and insulin tolerance tests were performed.

Results: Body weight loss was induced in food restricted groups (FR1 and FR2 group) compared to control group, with further body weight loss in FR1 group than FR2 group ($P<0.05$). The infiltrated adipose tissue immune cell population was increased in FR1 group but not in FR2 group compared with control group ($P<0.05$). The expression of MCP-1 and TLR-4 mRNA expression was increased in FR1 group but not in FR2 group compared with control mice ($P<0.05$). The expression of lipolysis related genes, adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), and lipoprotein lipase (LPL) mRNA was increased in FR1 group compared with FR2 group ($P<0.05$). The serum corticosterone levels were significantly increased in both FR1 and FR2 compared with control group ($P<0.05$), with much higher in FR1 group than FR2 group ($P<0.05$). After corticosterone infusion for 3 days, the expression of ATGL mRNA was increased in adipose tissue ($P<0.01$) and blood glucose levels was increased ($P<0.01$) and impaired insulin tolerance ($P<0.001$).

Conclusion: These results indicate that severe food restriction could induce stress, lipolysis, adipose tissue immune cell infiltration and inflammation, which in turn may influence insulin resistance.

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The effects of bariatric surgery on pancreatic lipid metabolism in morbidly obese subjects

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Background and aims: As a treatment modality for morbid obesity, bariatric surgery leads to a sustained weight loss and a high remission rate of type 2 diabetes. However, the exact mechanisms behind the improved glucose tolerance are not fully elucidated. In the present study we investigated whether bariatric surgery is able to improve pancreatic lipid metabolism in obese subjects.

Materials and methods: Totally, 24 morbidly obese subjects (BMI 41±4.0 kg/m², age 42±9.5 years) eligible for bariatric surgery, of which ten had type 2 diabetes, and 15 healthy age-matched subjects were recruited in the study. Pancreatic fatty acid (FA) uptake was measured at fasting state using PET/CT and a palmitate analogue [¹⁸F]FTHA. Pancreatic total volume, and fat and parenchymal volumes were measured using a CT based approach as recently described. Oral glucose tolerance test (OGTT) was performed to quantitate glucose tolerance and empirical/model-derived β -cell function parameters. Obese subjects were studied preoperatively and six months after bariatric surgery procedure (either Roux-en-Y gastric bypass or sleeve gastrectomy).

Results: Before surgery, obese subjects had higher rate of pancreatic FA uptake (1.4±0.6 versus 0.7±0.3 μ mol/min, $P < 0.001$) and fat percent (19±22 versus 4.0±4.0 %, $P < 0.01$), and had greater amount of ectopic fat (17±21 versus 3.0±3.1 ml, $P < 0.01$) than healthy controls. After bariatric surgery, obese subjects lost 24% of their weight ($P < 0.0001$), but were still obese (BMI 32±4.2 kg/m²). Fasting plasma glucose and HbA1c were lower (both by 12%, $P < 0.0001$), whereas no difference was found in plasma FFA levels (0.82±0.22 versus 0.76±0.18 mmol/l, NS). In subjects with type 2 diabetes preoperatively, parameters of β -cell function (insulinogenic index, glucose sensitivity, rate sensitivity) were improved (all $P < 0.02$), and in eight out of ten diabetes was in remission. In the obese group, pancreatic FA uptake (1.4±0.6 versus 1.0±0.4 μ mol/min, $P < 0.01$), fat percent (19±22 versus 14±13 %, $P < 0.01$), and ectopic fat volume (17±21 versus 11±12 ml, $P < 0.01$) were decreased, whereas parenchymal volume was unchanged (79±29 versus 72±22 ml, NS) after operation. Diminishment of ectopic fat was independent of weight loss and FA uptake (NS). Decrease in total pancreatic volume was associated with improved glucose tolerance, as measured with mean glucose during OGTT ($r = 0.59$, $P < 0.005$), and insulinogenic index ($r = -0.45$, $P = 0.04$).

Conclusion: The present study shows that, pancreatic lipid accumulation in obesity is reversible and modified by bariatric surgery. The results of the study imply that decreased pancreatic FA metabolism and loss of ectopic fat play a role in the improved glucose tolerance seen after bariatric surgery.

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The metabolic signature of RYGB is similar to that of equivalent caloric restriction

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Background and aims: Diabetes improves or remits within days following gastric by-pass surgery. It is debated whether this dramatic effect is due to the strict caloric restrictions imposed in the peri-operative period or due to surgery-specific changes in metabolism.

Materials and methods: Ten patients with type 2 diabetes underwent a strictly-observed 9-day inpatient caloric restriction intervention. Following re-equilibration to baseline characteristics (average 3.3 months), they were readmitted for a second identical intervention (identical daily oral intake) during which they also underwent gastric bypass. All measurements (6-hrs Mixed Meal Challenge Test) were performed before and after each study period (total of four measurements/patient). This precise study design allowed us to separate the effects of diet (first period) from the effects of surgery (second

period-first period) on the hormonal changes observed after surgery. Paired t-test was used to compare changes observed during each period.

Results: Patients were 54.1±/8.7 years old, 3 males, 70% minorities, BMI 50.8±/9 kg/m², with advanced diabetes (9.2±/8 years duration, treated with 1.5 oral agents, additionally 7 were also treated with insulin, median dose 0.8 u/kg). There was comparable and significant improvement in glycaemia, insulin sensitivity and beta-cell function. The diet only period fared better in regards to changes in ghrelin, GIP, and hepatic glucose output. Surgery induced large increases in GLP-1 and PYY (figure).

Conclusion: The severe caloric restriction employed immediately post gastric bypass surgery is primarily responsible for the rapid improvement in glycaemia. The increase in incretin hormones is specific to surgery, but does not seem to have a direct effect on glycaemia, insulin resistance, nor beta-cell function. Their effect, if any, is likely mediated through central mechanisms which control appetite and promote the long term adherence to such restricted caloric intake.

	Diet period				Surgery period			
	Baseline	End	p value		Baseline	End	p value	Between-periods p
WOMAN IR	3.74 (1.05-2.43)	1.22 (0.50-1.54)	0.04		1.51 (1.13-1.89)	0.68 (0.60-1.20)	0.02	0.69
Metabolic Index	4.79 (2.04-6.64)	6.72 (4.20-9.38)	0.01		5.44 (3.67-7.22)	9.97 (8.39-11.56)	0.04	0.27
Disposition Index	0.14 (0.05-0.23)	0.27 (0.11-0.42)	0.02		0.13 (0.08-0.18)	0.29 (0.13-0.46)	0.05	0.64
Postprandial HbA1c (mg/dl)	1.74 (1.30-2.18)	1.30 (1.13-1.49)	0.01		1.70 (1.48-1.94)	1.70 (1.48-1.94)	0.88	0.15
Glucose AUC (mg/dl*min)	73034.43 (58017.49-88051.37)	63447.47 (50131.35-76770.75)	0.02		68081.77 (53122.09-84644.45)	50470.06 (47941.76-51008.36)	0.15	0.56
Insulin AUC	7524.77 (5586.17-9463.37)	7888.64 (5505.10-10272.08)	0.63		6026.08 (4881.84-9372.11)	4259.26 (3262.00-5256.44)	0.05	0.1
C-peptide AUC	1701.09 (1123.64-2274.75)	2061.85 (1384.70-2738.06)	0.04		1613.40 (1081.74-2444.82)	1750.02 (1158.05-1953.98)	0.76	0.12
Ghrelin AUC (pg/ml*min)	23880.13 (2023.02-27755.13)	9433.75 (2064.25-38641.25)	0.73		27209.36 (2081.63-29786.44)	37829.13 (25020.46-39937.79)	0.01	0.04
Ghrelin AUC (pg/ml*min)	4944.50 (3229.31-7560.49)	1732.50 (178.54-2686.64)	0.01		5707.65 (1177-11401.53)	2856.25 (-338.72-4051.42)	0.06	0.63
De novo Ghrelin AUC (pg/ml*min)	13345.25 (9471.80-17218.70)	10334.05 (7034.86-13654.44)	0.01		12508.05 (9528.48-15491.22)	11300.22 (8135.55-13880.81)	0.37	0.25
GIP AUC	92810.83 (68621.44-117000.23)	154041.08 (92634.52-214447.05)	0.01		93030.72 (67728.78-118332.66)	85848.91 (56882.45-114955.37)	0.33	0.01
GLP-1 AUC	12940.09 (6445.11-19255.80)	14709.19 (8551.80-21001.50)	0.19		11040.40 (5023.29-16276.59)	22967.07 (14257.43-31557.17)	<0.001	0.04
PYY AUC	21795.48 (14759.76-28781.20)	23786.17 (17395.62-26176.71)	1		18881.29 (14230.77-23535.80)	48195.38 (33773.71-62657.05)	0.002	0.005

AUC - area under the curve, GIP-1 - glucagon like peptide 1, HGO - hepatic glucose output, GIP - gastric inhibitory polypeptide, PYY - peptide YY

Figure: Metabolic and hormonal changes following a period of caloric restriction alone versus identical caloric restriction along with gastric bypass surgery. Data are mean (95% CI).

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Immediate post-operative effects after a mixed-meal test and long-term effects on hormone expression in the alimentary limb in gastric-bypassed patients

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Background and aims: Gastric bypass surgery (GBP) is the most effective treatment for morbid obesity. Also, GBP results in rapid improvement in glycaemia long before significant weight loss. In the present study we aimed to 1) investigate the influence of pre-surgery caloric restriction with that of the immediate effect of GBP on glycemia and 2) assess long-term effects of GBP on gut hormone expression in the alimentary limb.

Materials and methods: Obese women (47±1 years) were subjected to a mixed meal test (MMT) four weeks before (MMT-4w), 4h before (MMT-4h) and one day after (MMT+1d) Roux-en-Y gastric bypass surgery. MMT-4w was performed before initiation of the low-calorie diet regimen and MMT+1d constituted the first meal the patients received after surgery. Blood was collected at -10, -5, 0, 5, 10, 15, 30, 60 and 90 minutes upon initiation of MMT-ingestion. Glucose, insulin, glucose-dependent insulinotropic peptide (GIP) and active glucagon-like peptide 1 (GLP-1) were analyzed for the MMTs. In another study, female patients were recruited and biopsies from the alimentary limb were obtained during surgery and via gastroscopy 12 months after GBP. Samples were analyzed for all major enteroendocrine cell populations using immunocytochemistry and morphometry.

Results: While there was difference in glucose levels between the various MMTs, the insulin response was markedly increased immediately at MMT+1d, compared to both the MMT-4w and MMT-4h (2.4-fold and 2.8-fold, respectively). Active GLP-1 levels were similar in all MMTs, whereas the GIP-response was higher at the MMT+1d, compared to MMT-4w and MMT-4h (1.6-fold and 1.4-fold, respectively). The elevation in insulin and GIP resulted in increased insulin AUC-to-GIP AUC-ratio ($p < 0.01$). Also, an immediate post-operative effect was observed on insulin AUC-to-glucose AUC ratio ($p < 0.05$). No differences were observed for any of the parameters analyzed when comparing MMT-4w and MMT-4h. One year after GBP, the alimentary limb of the patients displayed increased density of serotonin-immunoreactive (IR) cells (2.1-fold; $p < 0.05$) and GLP-1-IR cells (3.6-fold; $p < 0.01$), while there was no difference in villi length, density of ghrelin-IR cells, cholecystokinin-IR cells, secretin-IR cells, neurotensin-IR cells or GIP-IR cells.

Conclusion: Our data suggest that pre-surgery caloric restriction has no effect on the parameters analyzed; rather the re-arrangement of the gastrointestinal tract elicits an immediate stimulatory effect on insulin and GIP levels in response to a meal test. Our data also suggest that with this experimental

design, GIP is a strong contributor to the enhanced insulin secretion. Furthermore, we report for the first time the effects of GBP on expression of gut hormones in the alimentary limb one year after GBP. Our data showing that GLP-1 and serotonin immunoreactive cells are markedly increased in the alimentary limb 12 months after GBP provide anatomical prerequisites for sustained hormonal changes after GBP.

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Searching for biomarkers determining the early efficacy of bariatric surgery in the treatment of type 2 diabetes mellitus

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Background and aims: Bariatric surgery is an effective treatment for obesity and in 80–90% of subjects with type 2 diabetes improves significantly glycaemic control, independently of weight loss. Recent studies suggest different mechanisms that affects metabolic changes after surgery: incl. alterations in gut hormones secretion (ex. GLP-1), neurohumoral pathways modulation, changes in bile acid concentrations in the blood and/or altered intestinal microbiota. The recovery rate of the T2DM is also related to the method of bariatric surgery with the higher T2DM remission rate observed for a Roux-en-Y gastric bypass (RYGB) in comparison to a laparoscopic adjustable gastric banding (LAGB). The aim of this study was to search for the preoperative and postoperative metabolic biomarkers, which could determine the efficacy of the bariatric surgery and predict an early remission failure in the treatment of T2DM.

Materials and methods: The study was performed in the group of 49 obese patients with T2DM, who underwent bariatric procedures (laparoscopic sleeve gastrectomy - LSG or Roux-Y gastric bypass - RYGB) and were evaluated for an early remission failure (1 month after the surgery) of T2DM. The fasting serum samples were fingerprinted by LC-QTOF-MS and data were collected in ESI (+) mode (50–1,000 m/z). Chromatograms were aligned and quality assurance of obtained data was performed. Statistical analysis was conducted to compare patients before and one month after the surgery (paired t-test), and to compare (before the surgery) patients who recovered from T2DM in one month post-surgery with those who did not. Patients before the surgery were compared by use of t-test or Mann-Whitney test depending on the normality of variables distribution. Identification of significant features was performed based on custom library with accurate mass and retention time of more than 100 metabolites.

Results: We found that before bariatric surgery lower serum levels of lipid compounds (incl. lysophosphatidylcholines, phosphatidylethanolamines, sphingomyelins, ceramides) and lower vitamin D3 metabolites concentrations significantly predicted an early T2DM remission failure ($p < 0.01$ for all). One month after bariatric surgery, in all subjects with T2DM remission, the decrease in level of free lipids (p value < 0.05 – 0.01), acylcarnitines (p value < 0.04 – 0.008) and choline ($p = 0.0006$) was observed. On the other hand, in subjects without early T2DM recovery, no significant changes in acylcarnitines level were found.

Conclusion: Current results indicate, that changes in lipid metabolites contribute to the improvement of glycaemia and remission of the T2DM after bariatric surgery. The rate of regulation of fatty acid oxidation and mitochondrial function pathways may contribute to the time of the T2DM remission.

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PS 054 Inflammation and beta cell in type 2 diabetes

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IA-2(256-760), the only marker of islet autoimmunity that increases by increasing the degree of obesity in type 2 diabetes patients

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Background and aims: Approximately 5–6% of type 2 diabetes patients (T2DM), in Italian population, are positive for antibodies specific to type 1 diabetes (T1DM), although the same antibodies present significant quantitative difference compared to T1DM. Glutamic acid decarboxylase (GADs) is the marker with the highest prevalence, however also protein tyrosine phosphatase (IA-2IC) antibodies were shown to be an important autoimmune marker frequently found in these patients. We have previously demonstrated an extreme heterogeneity of T2DM positive patients in terms of phenotype, specifically the BMI varies widely. In the light of these findings we aim to evaluate whether the type and frequency of beta-cell specific antibodies vary according to different degrees of BMI in T2DM positive patients.

Materials and methods: In $n = 1850$ T2DM subjects recruited in central Italy from the Non Insulin Requiring Autoimmune Diabetes (NIRAD) study cohort of 5330, clinical and biochemical characteristics as well as the following antibodies were evaluated at recruitment: GADA, IA-2IC and IA-256-760. All patients were subdivided into three groups according to BMI: $BMI \leq 25$, $25 < BMI < 30$, $BMI \geq 30$. The differences of mean values of GADA, IA-256-760 and IA-2IC between groups were evaluated through the Anova and Benjamini-Hochberg test. A p value < 0.05 was considered statistically significant.

Results: Out of $n = 1850$ type 2 diabetes patients $n = 120$ (6.5%) were positive for at least one of the following antibodies: GADA (4.1%), IA-256-760 (3.3%) or IA-2IC (1.1%). As we consider the $n = 120$ T2DM antibody positive patients, GADA and IA-2 IC showed decreasing frequencies with the increase of BMI (p for trend 30 (80% of positive patients). Analyzing GADA, IA-256-760 and IA-2IC titers in all T2DM subjects subdivided according to BMI, we observed a decreasing mean values of GADA and IA-2IC titers with the increase of BMI (p for trend < 0.0001 and p for trend = 0.02, respectively). The mean values of IA-256-760 titer, instead, were similar between the three groups of BMI.

Conclusion: We conclude that the IA-2 (256-760) specifically identify autoimmunity in obese T2DM.

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Human adenovirus-36 is uncommon in type 2 diabetes and is associated with increased insulin sensitivity in adults in Sweden

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Background and aims: Human adenovirus-36 (Adv36) increases adiposity, but also upregulates distal insulin signaling *in vitro* in human adipose and muscle tissue and *in vivo* in the rodent independent of adiposity. Accordingly, healthy adults and children with antibodies against Adv36 had increased insulin sensitivity and reduced hepatic lipid accumulation. We hypothesized that Adv36 infection would be less frequent in individuals with type 2 diabetes or impaired glycemic control.

Materials and methods: Presence of antibodies against Adv36 was analyzed for association to type 2 diabetes or impaired glycemic control in a longitudinal population-based sample of well-examined adults ($n = 1734$). Indices of impaired glycemic control included oral glucose tolerance, and circulating fasting levels of glucose, insulin and insulin-like growth factor binding protein-1 (IGFBP-1).

Results: Adv36 seropositivity was more common in those with NGT (normal glucose tolerance) than in those with diabetes (females: OR=17.2, 95% CI=4.0–74.3, males: OR=3.5, 95% CI=1.8–6.7). Also, females with NGT had higher frequency of Adv36 seropositivity than females with prediabetes (impaired glucose tolerance or impaired fasting glucose, OR=1.8, 95% CI=1.1–3.1). Within the female prediabetes group Adv36 seropositivity was

associated with higher insulin sensitivity reflected by reduced HOMA-IR and increased IGFBP-1.

Conclusion: Adv36 infection associated with lower occurrence of type 2 diabetes and better insulin sensitivity in adults, particularly among females.

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AKT/JNK switch induces beta cell apoptosis as part of TLR3 signalling during Cocksackievirus infection

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Background and aims: Type 1 diabetes results from a sophisticated interplay between genetic factors, immune system and environmental factors. Among environmental factors, viruses, mainly enteroviruses such as members of the Cocksackie B virus family, have been suspected since long time to trigger diabetes. Intra-islet viral particles have been detected in pancreata from patients with T1DM and T2DM, and known to cause β -cell dysfunction in vitro, suggesting that a direct infection contributes to onset of diabetes. Nonetheless the mechanisms of a correlation between virus infection and diabetes progression are poorly understood. Clarification of the viral triggered β -cell dysfunction and apoptosis may provide an indication to develop preventive therapies. We previously identified Toll-like receptor 3 (TLR3) and Protein kinase R (PKR) as first defensive defensive line during coxsackie virus infection. In this study we asked the question of what is the contribution of PKR and TLR3 in β -cell apoptosis during CVB3 infection.

Materials and methods: Isolated human islets and CM cell line were infected with coxsackievirus serotype B3. Replication of CVB was confirmed by immunostaining of viral protein 1 (VP1) and titration of islet lysate. shRNA and siRNA were used to silence TLR3 and PKR in both human islets and CM cells. Plasmids encoding for TLR3 and TBK1 were used for overexpression experiments. Either dominant negative expression constructs or specific inhibitors were used to study the role of JNK and AKT in human islets. Islet protein expression and phosphorylation were analyzed by western blot. Binding of CVB ssRNA and dsRNA was investigated by immunoprecipitation-RT-PCR coupled assays.

Results: A time course analysis of CVB3 infection for downstream signaling showed AKT activation at 1h post-infection, which lasts for several hours. AKT was down-regulated at 24h post infection, when activation of pJNK, Caspase-3 and VP1 appeared in human islets. A possible correlation between AKT activation and PKR/TLR3 signaling was investigated. Knock down of PKR resulted in increased virus infection (VP1), JNK phosphorylation and activation of caspase 3, while AKT phosphorylation was PKR-independent. Lack of TLR3 had a pro-survival effect in CVB3 infected human islets leading to a reduced reduced JNK phosphorylation, caspase activation and VP1 as well as TBK1 phosphorylation. In contrast, overexpression of TLR3 enhanced AKT activation together with VP1 induction. Overexpression of TLR3/TBK1 further enhanced phosphorylation of AKT, viral replication and caspase activation suggesting pivotal role of AKT, enhanced by TLR3. AKT inhibition reduced viral replication, activation of caspase 3 and phosphorylation of JNK. Overexpression of DN-JNK led to a decrease in apoptosis indicated by the decreased level of cleaved caspase 3 but not of VP1. Treatment of human islets with the JNK inhibitor confirmed the protective effects of JNK inhibition.

Conclusion: In summary, our data show that CBV infections have a direct deleterious effect on β -cell survival, resulting from virus-induced apoptosis, and potentiated by the AKT/TLR3 signaling pathway. With our present data we provide novel targets towards protecting the β -cell during virus infection.

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DPP-4 inhibitors: anti-inflammatory effects on M1 activation

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Background and aims: Macrophages are a heterogeneous cell population which, in response to the cytokine milieu, differentiate in either classically activated macrophages (M1) or alternatively activated macrophages (M2).

This plasticity is essential to regulate inflammation, immune response and tissue remodeling and it may be a novel therapeutic target in inflammatory-based diseases such as atherosclerosis. Some recent evidence suggests that dipeptidyl peptidase 4 may play a role in in cardiovascular diseases and that its inhibition might be beneficial in curbing inflammation and atherogenesis. The aim of this study was to evaluate the anti-inflammatory effects of a DPP-4 inhibitor on classical macrophage activation.

Materials and methods: Monocytes were isolated from healthy donors' buffy coats. After differentiating into macrophages, cells were stimulated with IFN γ and LPS in order to induce M1 activation, in presence or in absence of 2 μ M of DPP-4 inhibitor (KR-62436, Sigma Aldrich) for 24 hours. The working concentration of DPP-4 inhibitor was selected after dose finding experiments. Resting macrophages (RM) -no added stimuli- were used as a control. The expression of DPP-4 was evaluated in each experimental conditions, both by real time PCR and cytofluorimetric assay. Key pro-inflammatory genes and chemokines (IL6, IL8, TNF-alpha, MCP-1, COX2, SOD2, SOCS1) were evaluated by real time PCR. Protein expression of IL-6 and TNF-alpha were evaluated by ELISA. Results were obtained by means of six biological replicates. DPP-4 activity is presently being assessed.

Results: M1 macrophages showed a significant up-regulation of DPP-4 mRNA compared to RM (p=0.015), which was unchanged by DPP-4 inhibition. These data were confirmed by cytofluorimetric analysis. M1 significantly up-regulated also the expression of pro-inflammatory genes SOCS1, IL-6, COX2 (by one hundred-fold), IL8, TNF-alpha (by fifty-fold), and MCP1, SOD2 (by ten-fold) compared to RM (p<0.05 or less). The addition of DPP-4 inhibitor significantly reduced the mRNA expression of IL-6, TNF-alpha, MCP1 and COX2 (p<0.05), restoring the same gene expression level as RM, except for COX2 that remained significantly higher (by five-fold, p<0.05). TNF-alpha and IL6 proteins resulted significantly up regulated in M1 compared to RM (by forty five-fold and six-fold respectively, p<0.01) and decreased by DPP-4 inhibition (p <0.05) although remaining significantly higher compared to RM (by three-fold, p<0.05). DPP-4 activity is currently under investigation in order to assess its role, if any, in anti-inflammatory effects.

Conclusion: This study shows that DPP-4 is overexpressed in classical activated macrophages, setting the stage for an involvement of DPP-4 in inflammatory processes. DPP-4 inhibition induced a significant reduction of the principal pro-inflammatory genes and proteins, but without affecting DPP-4 expression. These data suggest an anti-inflammatory effect of DPP-4 inhibitors that might be beneficial in the treatment of inflammatory related disease.

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In the long run hyperbaric oxygen therapy attenuates pro-inflammatory processes in streptozotocin induced diabetes in rats

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Background and aims: Hyperbaric oxygen therapy (HBOT) is frequently used in diabetic patients with ulcerated wounds; however its possible negative consequences raise questions. In our previous experiments we showed that weeks after HBOT cardiovascular functions of rats with streptozotocin induced diabetes were improved or unaltered. In the present study our aim was to examine the effect of HBOT on systemic oxidative-nitrative stress and cytokine production in the same setting.

Materials and methods: Wistar rats (15) weighing 300 g were administered with a single dose of 70 mg/kg streptozotocin to induce diabetes mellitus. 7 diabetic and 8 controls underwent one-hour long hyperbaric oxygen treatment (HBOT: 2.5 bar) 12 times (in 16 days). 6 controls and 8 diabetic rats remained untreated. Two weeks after the completion of HBOT series, heparinized blood plasma and lymphocyte samples were collected. Malonyl-dialdehyde assay (MDA) were performed on the plasma samples and nitrotyrosine immunostaining (NT) was done on lymphocyte smears. Altered cytokines were identified by Rat Cytokine Antibody Array (R&D). According to the results the following cytokines were selected for ELISA measurement: Cytokine-induced neutrophil chemoattractant 1 (CINC-1), lipopolysaccharide induced CXC chemokine (LIX/CXCL-5), tissue inhibitor of metalloproteinase 1 (TIMP-1). For data analysis Two Way ANOVA and Bonferroni's post hoc test were implemented.

Results: Without HBOT plasma MDA levels were significantly higher in diabetic rats compared to control animals (9.03 μ M [6.33, 12.69] vs. 21.56 μ M

[7.63, 34.18], $p \leq 0.01$), however this difference was abolished after HBOT (6.24 μM [5.08, 6.71] vs. 9.68 μM [6.47, 19.24], NS.). Leukocyte tyrosine nitration was elevated due to diabetes with (23.78 \pm 3.43% vs. 41.2 \pm 3.49%, $p \leq 0.01$) or without HBOT (20.44 \pm 4.08% vs. 38.1 \pm 4.12%, $p \leq 0.01$). The plasma levels of pro-inflammatory cytokines were increased in untreated diabetes (CINC-1: 69.1 ng/L [63.85, 74.63] vs. 137.1 ng/L [66.71, 343.8], $p \leq 0.01$; LIX: 282 ng/L [141.9, 382] vs. 430.8 ng/L [327, 604.4], $p \leq 0.05$), however due to HBOT this difference was ceased (CINC-1: 66.96 ng/L [61.3, 83.5] vs. 96.9 ng/L [70.73, 147.5], NS.; LIX: 245.3 ng/L [172.6, 340.3] vs. 346.8 ng/L [276.9, 774.6], NS.). The anti-inflammatory factor TIMP-1 was not altered by diabetes in the lack of HBOT (24.49 $\mu\text{g/L}$ [21.48, 26.41] vs. 27.79 $\mu\text{g/L}$ [22.18, 29.73], NS.), on the other hand after HBOT the plasma level of TIMP-1 was significantly higher in diabetic animals (20.76 $\mu\text{g/L}$ [18.43, 25.62] vs. 32.31 $\mu\text{g/L}$ [21.65, 38.43], $p \leq 0.01$).

Conclusion: According to our results two weeks after HBOT, the oxidative stress and pro-inflammatory cytokine production induced by experimental diabetes were abolished. Also the anti-inflammatory factor TIMP-1 is elevated as a response to HBOT in diabetic animals. On the other hand nitrate stress is not altered by the therapy. These findings suggest that HBOT is not just safe to use in diabetic patients, but may also have beneficial effect on their chronic subclinical inflammation.

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The anti-inflammatory protein NUPR1 (p8) generally enhances viability of pancreatic islets but does not prevent lipotoxicity

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Background and aims: Type 2 diabetes is closely related to obesity. According to the so-called “nutrition overflow hypothesis”, type 2 diabetes results from nutrition-induced local tissue inflammation leading to insulin resistance, insulin secretory dysfunction and finally pancreatic beta cell loss. Free fatty acids acting through toll-like receptors are key mediators of this lipotoxic inflammatory response. (1) We previously demonstrated that cocultured human bone marrow-derived mesenchymal stromal cells (hMSC-TERT) protect rat INS-1E beta cells from alloxan- and streptozotocin-induced injury. (2) We further demonstrated that mice with beta cell-specific overexpression of the intracellular protein NUPR1 maintained insulin secretion and storage during high fat diet-induced local low grade inflammation and intense inflammatory stress by insulinitis. Here, we investigated the anti-lipotoxic potential of (1) cocultured hMSC-TERT and (2) beta cell-specific *Nupr1* overexpression.

Materials and methods: For coculture, INS-1E were seeded in wells and hMSC-TERT were seeded in inserts with 1 μm pores to allow soluble factors but not cells to pass the membrane. Primary mouse islets were obtained from transgenic (Tg) mice with beta cell-specific *Nupr1* overexpression under the control of the rat insulin gene 1 promoter (RIP1). Non-transgenic littermates served as WT controls. Lipotoxicity was induced by palmitate. Viability of cells and islets was measured by MTS assay.

Results: We first evaluated by kill curve experiments that 24 h exposure to 0.13 mM palmitate inhibits the viability of INS-1E by 50%. Experiments were performed with 0.1 mM palmitate. (1) 24 h exposure to 0.1 mM palmitate reduced viability of INS-1E by 42%. Cocultured hMSC-TERT could not prevent this loss of viability. (2) Tg islets with ectopic NUPR1 demonstrated 1.5-fold enhanced viability compared to WT controls. However, ectopic NUPR1 did not reduce lipotoxicity since 24 h exposure to 0.1 mM palmitate reduced viability by 50% in both WT and Tg islets.

Conclusion: Despite their protective effects in other experimental settings, cocultured hMSC-TERT and also ectopic *Nupr1* did not prevent lipotoxicity. Ectopic NUPR1 overproduction generally enhances viability of Tg islets and thereby contributes to enhanced beta cell function during inflammatory tissue stress in Tg mice compared to WT controls.

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Protection of pancreatic beta cells in vitro and in vivo by NUPR1 (p8) protein during inflammatory diabetogenic stresses

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Background and aims: Type 2 diabetes is closely associated with obesity. Chronically enhanced circulating free fatty acids activate toll-like receptors and induce local inflammation leading to insulin resistance, insulin secretory dysfunction and finally pancreatic β -cell loss. However, many obese subjects do not develop diabetes. This observation suggests the existence of molecular defence systems that prevent β -cell degradation in non-susceptible individuals. Therefore, identification of molecular mechanisms that provide anti-inflammatory protection and control β -cell turnover are of key interest. The intracellular protein NUPR1 has been shown to reduce tissue damage during acute pancreatitis in *Nupr1*^{-/-} mice. The protein is also expressed in the endocrine pancreas. To investigate its potential protective role and cellular mechanisms in the under diabetogenic stress, we generated transgenic mice with β -cell-specific *Nupr1* overexpression under the control of the RIP1 promoter (Tg).

Materials and methods: We induced low-grade inflammation by feeding mice with high fat diet (HFD) and/or insulinitis by multiple low dose STZ injections. Glucose tolerance was evaluated by measuring non-fasting random blood glucose, intraperitoneal glucose tolerance test (ipGTT), intraperitoneal insulin tolerance test (ipITT) and serum insulin levels (ELISA). β -cell mass was determined morphologically. Islet inflammation was quantified by numbers of infiltrating CD45+ lymphocytes and NF- κ B activation (Western blot). Ex vivo isolated islets from untreated wild type (Wt) and Tg animals and from animals after 20 weeks of feeding with HFD were cultured for 16 days without treatment and subsequently exposed for 24 hrs to 10 ng/ml IL-1 β or 0.33 mM STZ were evaluated by glucose stimulated insulin secretion and content (GSIS), cleaved PARP (Asp214) for apoptosis (ELISA) and BrdU for cell proliferation.

Results: Immunohistochemically, we observed an islet enriched expression of NUPR1 in non-Tg mice. In vivo, Tg mice displayed improved glucose tolerance, improved insulin secretion and increased β -cell mass during HFD and/or insulinitis as compared to Wt controls. NUPR1-Tg islets also showed reduced lymphocyte infiltration and reduced NF- κ B activation. Ex vivo, Tg islets displayed greatly reduced apoptosis while insulin secretion and content was significantly enhanced compared to Wt islets during 16 days of culture or in response to 24 h exposure to IL-1 β or STZ. Further, Wt islets showed a massively reduced proliferation rate in response to diabetogenic injuries, whereas this was completely maintained in isolated NUPR1-Tg islets.

Conclusion: NUPR1 protein may be an important molecular mediator in the stress defence system of pancreatic β -cells. It exhibits potent protection of insulin biosynthesis, secretion and content during inflammatory pancreatic β -cell stress by reducing activation of NF- κ B and apoptosis and enhancing proliferation capacity as a possible mechanism to compensate for the diabetogenic decrease in pancreatic β -cell mass.

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Siglec F knockout impairs glucose stimulated insulin secretion in isolated mouse islets

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Background and aims: Type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) result from a decline in β -cell function and survival partly as a consequence of islet inflammation. The inflammatory signaling and the activation of immune cells are caused by secreted stimulators or via cell-cell interactions. A group of cell surface adhesion and signaling molecules, the Siglecs (sialic acid-binding immunoglobulin (Ig)-like lectins) mediate such interactions. In humans, Siglecs are differentially expressed in α - and β -cells and show protective effects on β -cell function and survival upon overexpression. In this study we investigated, if the knockout (KO) of the functionally relevant paralog Siglec F shows an effect on β -cell function in mice.

Materials and methods: To investigate the effect of the knockout isolated mouse islets were treated with diabetogenic conditions (2 ng/ml IL-1 β /1000 U/ml IFN- γ or 22.2 mM Glucose/0.5 mM Palmitate) for 3 days and subjected

Glucose stimulated Insulin secretion. Expression studies were carried out by FACS analysis and quantitative RT-PCR. For depletion of infiltrated macrophages isolated mouse islets were treated with Chlodronate containing liposomes for 48 hours.

Results: Knockout of Siglec F shows no effect under diabetogenic treatment, but significant lower Insulin release upon Glucose stimulation under control conditions ($p < 0.05$ versus wildtype). FACS analysis of dispersed wild type (WT) mouse islets showed Siglec F expression only in a very small cell population that did not occur in KO Islets. Quantitative RT-PCR demonstrated the low expression of mouse Siglecs in isolated Islets, which disappeared after depletion of macrophages with Chlodronate-liposomes. Chlodronate treated WT and KO islets show no difference in Glucose stimulated Insulin secretion. **Conclusion:** Siglec F knockout islets show impaired Insulin secretion due to altered interaction with infiltrating macrophages.

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The role of S100A8 and S100A9 in inflammatory interaction between pancreatic islets and macrophages

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Background and aims: The numbers of macrophages in the pancreatic islets of type 2 diabetes patients were reportedly increased. Yet, the pathophysiological significance of macrophages infiltration into islet is unclear. In this study, we focused on S100A8 and S100A9 (S100A8/A9) in islets induced by a combination of macrophage and free fatty acids. They are inflammatory proteins mainly expressed in monocyte and neutrophil. To address the mechanism of mutual interaction of islets with macrophage, we investigated the role of S100A8/A9 in this process.

Materials and methods: Isolated C57BL/6J mouse pancreatic islets were co-cultured with unstimulated peritoneal macrophages in the presence of lipopolysaccharide (LPS), palmitate, oleate, or adipocytes derived from epididymal fat, *in vitro*. To study the effect of S100A8/A9 on beta cell function, we examined glucose-stimulated insulin secretion in S100A8- and S100A9-adenoviral-overexpressed islets. Furthermore, we also analysed S100A8/A9 expressions in islets from obese diabetic db/db mice. To elucidate the effect of S100A8/A9 on macrophages, macrophages were stimulated with medium used for co-culturing islet and macrophages.

Results: In the presence of macrophages, the mRNA expressions of IL-1 β , TNF- α , IL-6, CCL2, S100A8, and S100A9 in islets were increased (2.3-fold**, 2.9-fold**, 7.3-fold**, 9.6-fold**, 13.3-fold** and 72.7-fold**). LPS enhanced the macrophages-induced expression of TNF- α , IL-6 and CCL2 in islets (1.7-fold*, 2.4-fold** and 2.3-fold**), whereas palmitate augmented the expression of S100A8 and S100A9 under the same conditions (4.8-fold** and 3.6-fold**). Oleate didn't affect those gene expression levels in islets. Neither LPS nor palmitate was sufficient to facilitate S100A8/A9 expressions in islets in the absence of macrophage. When islets were co-cultured with adipocytes, the expressions of IL-1 β , TNF- α , IL-6 and CCL2, S100A8 and S100A9 in islets were tended to elevate. The induction of TNF- α , S100A8 and S100A9 expressions by adipocytes in islets were up-regulated by the addition of macrophages (2.4-fold**, 2.3-fold** and 4.8-fold**). The expression levels of S100A8 and S100A9 were potentiated at high glucose concentration (22.2 mM) compared with those at low glucose concentration (5.6 mM) in the presence of palmitate (7.3-fold** and 4.6-fold**). Immunoblot and ELISA suggested S100A8/A9 were secreted from co-cultured islets to supernatant. Adenoviral-overexpression of S100A8/A9 in islets didn't affect insulin secretion. Immunohistochemical analysis indicated that only beta cells, but not alpha cells or acinar cells, expressed S100A8/A9 in pancreas. In islets from db/db mice, the expression of IL-1 β , TNF- α , IL-6, CCL2, S100A8, and S100A9 in islets were higher than that in islets from db/+ mice (6.1-fold**, 2.6-fold**, 19.4-fold**, 7.7-fold**, 18.2-fold* and 10.7-fold†, respectively). Culture supernatant from islet, macrophage, and palmitate up-regulated the expression level of IL-6 in unstimulated peritoneal macrophages (2.3-fold**). ($\dagger p < 0.1$, * $p < 0.05$, ** $p < 0.01$)

Conclusion: These results suggest that S100A8/A9 proteins in beta cells were enhanced by macrophages, free fatty acids and high glucose, like diabetic condition. Secreted S100A8/A9 were thought to trigger the inflammatory cytokine expression in macrophages. Taken together, S100A8/A9 may mediate the inflammatory interaction between pancreatic islets and macrophages in the progression of diabetes.

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Angiotensin II causes pancreatic islet dysfunction and inflammation independently of vasoconstriction

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Background and aims: Pathological activation of the renin-angiotensin system (RAS) is closely associated with the metabolic syndrome and new-onset of type 2 diabetes (T2D) can be delayed by RAS inhibition. In animal models of T2D RAS blockage enhances glycemic control and pancreatic islet function. The aim of this study was to investigate the effects of angiotensin II on islet function and underlying mechanisms, independently of its effects on blood pressure.

Materials and methods: For *in vitro* experiments, angiotensin II-treated human or mouse pancreatic islets and the rat beta-cell line INS-1E were used for glucose-stimulated insulin secretion assays, RNA extraction or protein measurements. *In vivo* studies were done with C57BL/6J mice fed a high fat diet (HFD) for 12 weeks followed by implantation of angiotensin II-releasing osmotic pumps. Hydralazine was given in the drinking water to avoid vasoconstriction. After 4 weeks, at the end of the study, intraperitoneal glucose or insulin tolerance tests were performed and heart blood and organs were taken for further analysis.

Results: Treatment of human islets with angiotensin II significantly increased the expression of the pro-inflammatory cytokines IL-1 β , IL-6 and the chemokine MCP-1, caused beta-cell apoptosis (increase 3.1-fold \pm 1.34) and impaired glucose-stimulated insulin secretion (stimulatory index reduced by 23.7%, $p < 0.005$). The angiotensin II-induced upregulation of IL-6 was inhibited by an IKK-2 inhibitor and by the IL-1Receptor antagonist IL-1Ra. Similarly angiotensin II reduced the stimulatory index of glucose-stimulated insulin secretion in mouse islets (31.9% reduction) and INS-1E cells and up-regulated IL-6 levels (1.49-fold \pm 0.25). *In vivo* cotreatment with angiotensin II and hydralazine normalised the blood pressure. Glucose tolerance testing revealed impaired glucose homeostasis in angiotensin II- and hydralazine-treated HFD mice compared to saline- or hydralazine-treated HFD mice. This was associated with impaired insulin secretion whereas insulin sensitivity was unchanged. Further, animals revealed increased circulating and islet-derived IL-6 levels (2.28-fold increase vs. hydralazine group). Treatment with an anti-IL-1 β antibody rescued the phenotype of the angiotensin II- and hydralazine-treated mice by restoring glucose-stimulated insulin secretion along with a reduction of islet-associated immune cells.

Conclusion: We conclude that chronic local RAS activation leads to islet dysfunction *in vitro* and *in vivo* and independently of vasoconstriction. Further, the rescue with NFkB inhibition and IL-1 blockage suggests that inflammation promotes angiotensin II-mediated islet dysfunction.

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PS 055 Inflammation in adipose tissue and muscle

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Lycopene attenuates the inflammatory adipose tissue response to high fat feeding by regulating both macrophage recruitment and M1/M2 status

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Background and aims: Obesity activates the innate immune system with subsequent recruitment of immune cells such as macrophages and T cells, which contribute to the development of insulin resistance. In particular, adipose tissue macrophages (ATM) recruitment and polarization are considered pivotal in obesity-induced inflammation and insulin resistance. However, promising treatment modalities targeting ATM for insulin resistance and type 2 diabetes remain limited. Here, we show that lycopene, an antioxidant carotenoid compound, ameliorates adipose tissue inflammation and whole-body insulin resistance in high-fat diet (HFD)-induced obese (DIO) mice by regulating both macrophage recruitment and M1/M2 status.

Materials and methods: C57BL/6J mice were fed a HFD or a HFD containing lycopene (HFD+LY; 12 mg/kg body weight) for a total of 8 weeks. The histology of epididymal white adipose tissue (eWAT) and insulin sensitivity were examined. Next, we quantified immune cells in stromal vascular fraction of eWAT, peripheral blood, and bone marrow by fluorescence-activated cell sorter (FACS).

Results: After 8 weeks of feeding, lycopene improved HFD-induced glucose intolerance, and hyperinsulinemia (HFD 4.0 ± 0.2 vs HFD+LY 2.0 ± 0.1 ng/ml, $p < 0.01$; fed state), and also enhanced insulin signaling assessed by IR β and Akt phosphorylation in eWAT of DIO mice. HFD+LY mice had decreased macrophage infiltration and crown-like structure formation in eWAT compared with HFD mice even though weight and adiposity were similar. In addition, lycopene administration decreased expression of mRNA for MCP-1 by 50% and pro-inflammatory cytokines, such as TNF α and interleukin-1 β , by 62% and 55%, respectively (all $p < 0.01$) in eWAT of DIO mice. DIO mice had 2.3-fold increase in the levels of thiobarbituric acid reactive substances (TBARS) in eWAT compared to wild type (WT) mice, whereas lycopene reduced lipid peroxidation by 50% ($p < 0.01$) in DIO mice. These findings were associated with reduction of ER stress (CHOP/GRP78), and attenuation of JNK/p38MAPK and NF- κ B activation in eWAT. To further assess the impact of lycopene on adipose tissue inflammation, FACS analysis was performed on stromal vascular cells isolated from eWAT. ATMs identified as CD45 $^{+}$ CD11b $^{+}$ F4/80 $^{+}$ cells were increased in DIO mice by 11.4-fold compared with WT mice. In addition to reduction of total ATM content, HFD+LY mice had 35% fewer CD11c $^{+}$ CD206 $^{+}$ (M1) ATMs whereas 60% more CD11c $^{+}$ CD206 $^{+}$ (M2) ATMs than HFD mice, resulting in predominance of M2 over M1 ATM population. However, the predominance of the Ly6C hi over Ly6C lo monocyte population was not observed in both peripheral blood and bone marrow of HFD+LY mice. Moreover, the numbers of either CD3 $^{+}$, CD4 $^{+}$, or CD8 $^{+}$ T cells were decreased by 36%, 52%, or 38% respectively (all $p < 0.05$) in eWAT of HFD+LY group compared with HFD mice. In parallel, lycopene (10–50 nM) suppressed LPS-induced M1 markers mRNA expression (TNF α , MCP-1, and RANTES) in Raw264.7 macrophage cells whereas it augmented IL-4-induced M2 markers mRNA expression (IL-10 and Arg1) in a dose-dependent manner.

Conclusion: Lycopene, a potent antioxidant, decreases ATM recruitment and causes a dynamic M2 dominant shift of ATM, and thereby attenuates obesity-induced inflammation and insulin resistance. Reduction of oxidative stress might be a relevant strategy to limit inflammatory adipose tissue response and subsequent development of insulin resistance.

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CD11c $^{+}$ adipose tissue macrophages are bone marrow-derived proliferating cells with a mixed polarization phenotype that accumulate early in obesity

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Background and aims: Accumulation of pro-inflammatory (M1) macrophages in adipose tissue (AT) is a key event in obesity. These adipose tissue macrophages (ATMs) express CD11c (CD11c $^{+}$), in contrast to resident CD11c $^{-}$ (M2) ATMs. ATMs have been shown to be capable of proliferating. However, the exact dynamics and relative contribution of recruitment and local proliferation is unknown. We aimed to identify the origin of CD11c $^{+}$ ATMs and investigate the temporal dynamics of ATM accumulation.

Materials and methods: We created mice expressing CD45.2 on resident immune cells and CD45.1 on bone marrow-derived immune cells by a bone marrow transplantation strategy. After 6 weeks recovery, the mice were given a control or high fat diet (HFD) for 10 days or 5 weeks. Stromal vascular fraction (SVF) of epididymal AT was analysed and sorted with flow cytometry. qPCR was performed on sorted ATMs to measure proliferation and activation markers. Data were analysed using t-tests or 1-way ANOVA.

Results: In lean mice, virtually all CD11c $^{+}$ ATMs were from donor origin, while 20% of the CD11c $^{-}$ ATMs remained from acceptor origin. Upon HFD, CD11c $^{+}$ ATMs already increased after 10 days (14.84 ± 0.46 vs $12.22 \pm 0.95\%$ SVE, $p < 0.05$) and further accumulated until 5 weeks (18.43 ± 0.93 vs $12.22 \pm 0.95\%$ SVE, $p < 0.001$). CD11c $^{-}$ ATMs were only increased after 5 weeks of HFD (26.59 ± 0.88 vs $18.25 \pm 1.28\%$ SVE, $p < 0.001$). In line, monocytes are also increased after 10 days of HFD (10.31 ± 0.88 vs $6.59 \pm 0.55\%$ SVE, $p < 0.001$) and levels remain constant after 5 weeks. Flow cytometry showed that only CD45.1 cells were accumulated upon HFD (14.40 ± 0.44 vs $11.81 \pm 0.89\%$ SVE, $p < 0.05$) while resident CD45.2 cells were not affected. qPCR analysis of sorted ATMs revealed that M1 CD11c $^{+}$ ATMs display high TNF and Arginase-1 expression while expression of Mannose Receptor and MCP1 was lower compared to the putative M2 CD11c $^{-}$ ATMs. Additionally, HFD only marginally affected gene expression of both M1 and M2 markers. Moreover, only CD11c $^{+}$ ATMs displayed an increased expression of the proliferation marker Ki67 upon HFD compared CD11c $^{-}$ M2 ATMs.

Conclusion: Our data show that monocytes numbers and CD11c $^{+}$ “M1” ATMs accumulate in the early phases of obesity suggesting that recruiting monocyte precursors of CD11c $^{+}$ M1 ATMs from bone marrow is an early event in obesity. Moreover, these ATMs have increased Ki67 expression suggesting that local proliferation of recruited cells contributes to accumulation in obese AT. Interestingly, ATM-subtypes have mixed M1/M2 profiles and obesity affects cell numbers rather than inflammatory phenotype.

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Adipocyte hypertrophy, inflammation and fibrosis: early changes in subcutaneous adipose tissue in healthy, non-obese subjects predisposed to type 2 diabetes

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Background and aims: Adipose tissue dysfunction is thought to be an important player in the development of type 2 diabetes. We investigated adipocyte hypertrophy, impaired adipocyte differentiation, inflammation, remodeling and adipose tissue fibrosis in healthy, non-obese, genetically predisposed subjects compared with control subjects with no known genetic predisposition for type 2 diabetes.

Materials and methods: We recruited 17 healthy, non-obese first degree relatives (FDR) of persons with type 2 diabetes and 17 control subjects. Groups were matched for gender and BMI. Glucose tolerance was determined by an OGTT and insulin sensitivity was calculated using HOMA-IR. Blood samples were collected and subcutaneous abdominal adipose tissue biopsies obtained for gene expression analysis and adipocyte cell size measurement.

Results: In spite of similar age, gender, BMI or fat percent in the groups, FDR displayed higher waist/hip ratio, fasting serum insulin levels, HOMA-IR and serum triglycerides, as well as adipocyte hypertrophy (all p -values < 0.05). Adipocyte hypertrophy in the FDR group, but not among the controls, was

associated with measures of impaired glucose metabolism. There was an increased wnt-signaling activity in the subcutaneous adipose tissue of the FDR and the gene expression of CCND2, Tcf7L2 and FN1 were significantly up-regulated. Cytokines involved in inflammation (TNF α , IL1 β and IL10) showed an increased expression in adipose tissue among the FDR, as well as markers of macrophage infiltration (CD68 and MCP1). There were also signs of adipose tissue remodeling (MMP2) and fibrosis (CTGF and ACTA2) among the FDR.

Conclusion: Genetic predisposition for type 2 diabetes is associated with impaired glucose metabolism and adipose tissue dysfunction, including inflammation, differentiation and remodeling. Reduced ability to maintain adipose tissue functionality may be a major susceptibility factor for later development of type 2 diabetes.

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Evidence for the presence of the phagocytic machinery in adipose tissue

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Background and aims: The chronic inflammatory state that develops in adipose tissue (AT) during obesity is generally considered to promote insulin resistance (IR) and Type 2 Diabetes (DMII). Obese, inflamed AT is characterized by the presence of dead hypertrophic adipocytes surrounded by inflammatory macrophages, forming so-called Crown-like structures (CLS). Contrary, in lean AT hardly any dead adipocytes and inflammatory macrophages can be identified. Immunologically silent clearance of dead cells by macrophages is known to be crucial for maintaining homeostasis in many tissues. The presence of CLS in obese, inflamed AT suggests that the clearance of dead adipocytes is disrupted during the development of obesity and might contribute to AT inflammation. We therefore aim to unravel the metabolic and immunologic (dys)regulation within AT macrophages (ATM) related to their phagocytic trait, in order to find new leads for therapeutic targets and strategies for obesity-induced AT inflammation and subsequent IR and DMII.

Materials and methods: In vivo, C57Bl/6 mice were fed a Low Fat diet (LFD) or a High fat diet (HFD) to promote IR and DMII. Phagocytosis was studied ex vivo using a co-culture system of labelled macrophages and dead adipocytes followed by staining for lipids and subsequent analysis using Flow Cytometry. The effect of AT-derived factors on the phenotype of the macrophages was examined using a co-culture system with AT explants. Quantitative PCR, Western Blotting, ELISA and Microarray were used to determine the immunological and metabolic state of macrophages.

Results: Microarray analysis of AT depots isolated from mice on a LFD or HFD revealed differential regulation of multiple genes that are known to play crucial roles in phagocytosis (Mertk, Gas6, Mfge8, Lrp, Gulp1, Elmo1 and Ucp2) upon the development of obesity, accompanied by changes in the expression of apoptosis- (Caspases, Bax) and inflammation-related (IL-1 β , Nlrp3) genes. In vitro analysis demonstrated a profound increase in lipids in macrophages that were co-cultured with dead adipocytes (using Flow Cytometry), suggestive of phagocytosis of adipocytes by macrophages. Upon the phagocytosis of dead adipocytes, genes involved in fatty acid oxidation (FAO; Ppara, Pgc1 α) were higher expressed and the macrophages were skewed towards a predominant anti-inflammatory phenotype, marked by higher expression and secretion levels of IL-10 and decreased expression and secretion of TNF α . Interestingly, macrophages that were co-cultured with AT explants from lean mice had a twofold higher phagocytic capacity than macrophages that were cultured without AT explants. An increased expression of genes involved in phagocytosis (Mertk, Gas6, Ucp2) in these macrophages was accompanied by higher expression levels of genes involved in FAO (Cpt1 β , Acad, Pgc1 β) and anti-inflammatory cytokines (IL-10); closely mimicking the phenotypic changes that were found in macrophages that have phagocytised dead adipocytes.

Conclusion: Our data suggest that the phagocytic machinery is present in AT and is differentially regulated in obese, inflamed AT. Interestingly, macrophages are capable of phagocytising dead adipocytes *ex vivo* and their phagocytic capacity is enhanced by AT-derived factors.

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Local biocommunication between peripancreatic adipose tissue and pancreatic beta cell: characterisation of factors involved in beta cell dysfunction

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Background and aims: In metabolic syndrome, pancreatic beta-cell is able to compensate for the insulin resistance by increasing beta-cell mass and function which allows to maintain the normoglycaemia. During the transition from pre-diabetes/obesity to type 2 diabetes (T2D), beta-cell is subjected to metabolic and inflammatory stress, that gradually lead to secretory dysfunction. In obesity, factors secreted by visceral adipose tissue may influence beta-cell mass and function. Moreover, increased visceral adiposity is associated to the development of peripancreatic adipose tissue (PPAT), which penetrates the pancreas and establishes direct contact with islets of Langerhans. Rebuffat et al (Endocrinology, 2012) showed in a model of obesity and insulin resistance that factors secreted by the PPAT modulate the proliferation of beta-cells. This is in favor of a local biocommunication between PPAT and beta-cell. Our goal is to characterize the PPAT in terms of immune cells and inflammatory factors secreted during the onset of type 2 diabetes (T2D) and determine how these factors are able to influence beta-cell compensation and decompensation in response to insulin resistance and metabolic syndrome.

Materials and methods: Our study was performed in the Zucker fa/fa rats/ZDF rats model of obesity and T2D. Three groups of animals were studied, pre-obese rats (6 weeks old) obese/pre-diabetic rats (10 weeks old) and diabetic rats (12 week olds). The presence of immune cells infiltrating the PPAT was investigated by immunohistochemistry using the macrophage marker ED1. The expression profile of peripancreatic fat-derived factors was analyzed by antibodyarray (Chemiarrray, RD System) and proteomic technology. A comparison of each group of rats allowed us to identify modifications in adipose tissue-derived factors and to correlate these changes to the metabolic status of the animals.

Results: An increase in macrophages infiltrating the PPAT was observed in obese/pre-diabetic animals (10 weeks old). Using chemiarray technology, we observed changes in cytokines and chemokines profiles secreted by PPAT in the ZDF rat model. While in the early stages of the disease we observed an increase in pro-inflammatory cytokines and chemokines secreted by PPA (such as, CINC- 1, 2, 3, IL-1 β , LIX, TIMP-1, and MIP- 1 α), surprisingly and unexpectedly, the expression profile of these factors changed and their levels decreased, when animals become diabetic (12 weeks ZDF rats). Proteomic profile also varies during the onset of the disease.

Conclusion: Therefore, our results showed that in obese/pre-diabetes rats, PPAT adipose tissue expression of inflammatory cytokines and chemokines are modified and thus could (i) induce and perpetuate inflammation by attracting immune cell in tissue and (ii) contribute to progressive beta-cell dysfunction occurring in T2D.

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The metabolic and anti-inflammatory effects of long-term resveratrol intake; a randomised clinical trial

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Background and aims: Low-grade inflammation due to macrophage infiltration and hypoxia in adipose tissue (AT) is thought to be the link between obesity and the metabolic syndrome (MS). Resveratrol (RV) is a natural anti-inflammatory compound found in e.g. grape-skin that reduces inflammation and normalizes both insulin sensitivity and lifespan of diet induced obese rodents. Sirt1 enzymes, thought to mediate the RV-effects in rodents, are present in human AT. In vitro, RV stimulates human AT to increase lipolysis, reduce secretion of the macrophage-attracting MCP1 and reduce inflammation. Furthermore, hypoxia induced inflammation in AT is completely reverted when exposed to RSV. In clinical trials the effects of RV are inconclusive. A previous short term cross over study has shown that RV lowered systolic blood pressure, hepatic liver fat, and plasma triglycerides, leptin, TNF- α , leukocytes, alanine transaminase (ALT) and HOMA index in healthy obese humans. However, we and others have not been able to verify these findings. We aim to investigate the anti-inflammatory effects of long term RV intake and it's potential to ameliorate aspects of MS.

Materials and methods: A placebo controlled double blind clinical trial. 76 males with MS were randomized to treatment with high dose RV (HD) 500 mg x 2 or low dose RV (LD) 75 mg x 2 daily or placebo (PL) for 4 months. Before and after treatment, biopsies from skeletal muscle, adipose tissues were obtained for analysis of gene expression. MR and DEXA-scans were used to quantify changes in body fat amount and body composition respectively. Clinical blood pressure (BP) measurement, blood tests and urine samples were obtained at day 0, 30, 60 and 120 to follow BP, inflammation, lipid profile, liver function, insulin sensitivity (HOMA).

Results: 76 were randomized for the trial and 66 completed the four months of treatment. At baseline the subjects were well matched for BMI, age and waist circumference. However lean mass was significantly greater in LD compared to HD ($p=0.048$) and systolic BP was significantly higher in the PL group compared to LD group ($p=0.05$). Plasma biochemistry: At baseline there was a positive correlation between severity of metabolic syndrome and IL-6 level ($p<0.03$). Metabolic effects: There were no significant effects of RV treatment on total cholesterol, high-density lipoprotein (HDL), triglyceride, ALT, insulin, glucose (or HOMA-IR). Anti-inflammatory effects: There were no changes in hs-CRP, IL-6, soluble urokinase-type plasminogen activator receptor (suPAR) in plasma, and there were no significant changes of IL-6 and TNF- α gene expression in AT or muscle tissue between the groups. Body composition: There was no difference in BMI, total lean mass, total fat mass, total fat percentage, subcutaneous adipose tissue, visceral adipose tissue, liver fat or extra/intra myocellular lipid between the groups. BP was unchanged between the groups.

Conclusion: Increasing severity of metabolic syndrome is associated with higher IL-6 plasma levels. However, RV did not affect IL-6 levels. So neither long term HD or LD treatment affects low-grade inflammation, or parameters of the MS. RV does not change body composition or AT depot size irrespective of dose. We conclude that RV has no role in reducing low-grade inflammation as seen in MS and shows no promise in ameliorating any aspects of the MS.

Clinical Trial Registration Number: NCT01412645

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Elevated glucose concentrations induce Prep1 overexpression through epigenetic modifications

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Background and aims: Changes in epigenetic regulation of gene expression represent potentially important pathogenic mechanisms behind complex diseases such as diabetes. High glucose concentrations that mimics diabetic conditions can induce *in vivo* chromatin remodeling and histone modifications to allow increased binding of key transcription factors, such as the nuclear factor κ -B (NF- κ B), regulating inflammatory and diabetes-related genes. *Prep1*, a homeodomain transcription factor essential in embryonic development, has a role in glucose and energy metabolism. Adult *Prep1*-hypomorphic mutant mice (*Prep1*^{hi}), which express 2 to 3% of *Prep1* mRNA and up to 10% of the protein, exhibit enhanced sensitivity to insulin action and are protected from developing streptozotocin-induced diabetes. Consistent with these findings overexpression of the wild-type *Prep1* cDNA in L6 cells determined a >70% inhibition of insulin-stimulated 2-DG uptake by these cells and a similar decline in the expression of GLUT4 protein and mRNA. However, how the environment affect *Prep1* gene remains unknown. The aim of this study is to evaluate whether and how a diabetic condition such as high glucose can dysregulate *Prep1* gene expression by inducing epigenetic changes through NF- κ B recruitment to its promoter.

Materials and methods: We mimicked diabetic condition by culturing L6 skeletal muscle cells in High Glucose (25 mM) relative to Normal glucose (5.5 mM). *Prep1* and NF- κ B expression were analyzed by Real-Time PCR and western blot analysis. Chromatin Immunoprecipitation assays have been performed to identify histone marks and histone-associated proteins.

Results: L6 cells cultured in HG for 72 hours show a significant increase of *Prep1* mRNA and protein levels compared to NG cultured cells. Time course experiments of HG showed an increase of *Prep1* expression both at mRNA and protein levels yet at four hours of HG incubation with the maximum effect reached at 48 and 72 hours of high glucose incubation. This effect was specific to glucose since equimolar amounts of xylose (osmolarity control) and 2-DG had no effect. As expected *Glut4* mRNA levels decreased in re-

sponse to HG. In parallel, HG incubation induced nuclear translocation and binding to *Prep1* promoter of the transcription factor NF- κ B p65 that in turn enhanced the binding of the histone methyltransferase SET7/9 and histone acetyl-transferase p300 to *Prep1* promoter. This event coincided with an increase in lysine 4 dimethylation and lysine 9 and 14 acetylation on histone H3 (two marks of active transcription). Co-incubation of L6 cells with HG plus JSH-23, an NF- κ B inhibitor, prevented HG-induced p65 binding, histone modifiers recruitment and histone modifications on *Prep1* promoter and restored HG-induced *Prep1* overexpression.

Conclusion: Elevated glucose concentrations alter the epigenetic state of *Prep1* gene resulting in an increase of its expression levels. These findings might have clinical relevance as preliminary evidence in our lab indicates that *Prep1* gene is overexpressed in euglycemic offspring of type 2 diabetic patients, suggesting that *Prep1* overexpression may provide an early contribution to disease in these individuals. Clarify *Prep1* epigenetic regulation can yield new insights into the physiopathology of glucose homeostasis and uncover potential targets for diabetes treatment and prevention.

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Palmitate challenged muscle cells attract monocytes through TLR4-dependent release of ATP

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Background and aims: Obesity and type 2 diabetes are associated with chronic activation of the immune system, and insulin-resistance arises by both lipotoxicity and low-grade inflammation. In response to high fat diet, the fat tissue attracts immune cells that cause low, sustained inflammation responsible for making the body resistant to insulin. Although inflammation is clearly recognized in adipose tissue, recent studies show that high fat-feeding is also responsible for a significant increase in pro-inflammatory cytokine expression in muscle. Yet, it is unknown whether there is a cause-effect relationship between muscle inflammation and whole body insulin resistance. Strikingly, muscle contains resident macrophages and we and others have recently detected infiltrating pro-inflammatory macrophages in muscle from type 2 diabetic individuals or from high fat-fed mice. Beyond this, little is known about the interplay between immune and muscle cells in the context of hyperlipidic environment, and a key unresolved issue is whether and how muscle cells themselves are capable of attracting monocytes.

Materials and methods: Skeletal muscle is composed primarily of muscle fibers and satellite myoblasts, but also encompasses blood and lymph vessels, nerves and immune cells. As each of these cell types can potentially respond to a high fat environment *in vivo*, we chose a cell culture approach to investigate the specific crosstalk between muscle cells and macrophages in the context of fatty acid exposure. This strategy enables us to control individual variables, to determine vectorial communication, and to explore separately the responses of each cell type. Medium collected from L6 myotubes (conditioned media, CM) exposed to saturated or unsaturated fatty acids (palmitate vs palmitoleate) was tested for its ability to attract monocytes. Secreted factors in the CM and inflammatory pathway activation in myotubes were analyzed using western blot, qPCR, nucleotide degradation strategies and gene knockdown.

Results: CM from L6 myotubes treated with palmitate - but not palmitoleate - induced THP1 monocyte migration across transwells. Palmitate caused both myotube lipotoxicity and inflammation. Although ceramide levels rose, they were not required for monocyte chemoattraction. Palmitate activated the TLR4-NF κ B pathway in myotubes and elevated cytokine expression, but the monocyte chemoattracting agent in CM was not a polypeptide. Instead, nucleotide degradation eliminated the chemoattracting properties of CM. Moreover, palmitate induced the expression and activity of pannexin-3 channels in myotubes, mediated by TLR4-NF κ B. Importantly, TLR4-NF κ B inhibition or pannexin-3 knockdown in myotubes prevented monocyte chemoattraction by CM.

Conclusion: These findings constitute proof of concept that high levels of saturated fats may promote monocyte migration towards skeletal muscle *in vivo*, promoting the macrophage infiltration of this tissue that arises with fatty diets. They also predict that targeting chemokine production may be insufficient to reduce macrophage infiltration of muscle, as other factors such as nucleotides may significantly contribute to immune cell chemoattraction. Moreover, this study identifies pannexin-3 as an interesting target to taper the arrival and contribution of tissue inflammatory macrophages to metabolic disease.

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PS 056 Novel adipokines

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Serum omentin is associated with lipid levels, but not with glucose tolerance and type 2: KORA F4 study

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Background and aims: Circulating omentin levels are positively correlated with adiponectin. Although several studies suggested that omentin - like adiponectin - may be inversely associated with obesity and insulin resistance (HOMA-IR), data from population-based studies are not available so far. Therefore, we investigated in the population-based KORA F4 survey from Augsburg/Germany (i) whether serum levels of omentin were associated with prediabetes, type 2 diabetes (T2D) and continuous T2D risk factors and (ii) whether associations between omentin, prediabetes, T2D and related risk factors were mediated by BMI.

Materials and methods: Serum levels of omentin were measured by ELISA in 1089 participants (48% male; age range 61–82 years; BMI 29 ± 4 kg/m²; NGT: n=576, IFG: n=56, IGT: n=186, IFG/IGT: n=46; newly diagnosed T2D: n=66, known T2D: n=159). Associations between serum omentin, glucose tolerance and continuous risk factors of T2D were assessed using logistic and linear regression models, respectively.

Results: We found no inverse association between serum omentin and prediabetes or diabetes ($P > 0.05$ for unadjusted comparison of categories of glucose tolerance) and no significant associations with glucose tolerance status after adjustment for age, sex, BMI, lifestyle factors, lipids, hypertension and history of myocardial infarction. After adjustment for multiple confounders, serum levels of omentin were associated with blood lipids (HDL cholesterol: $\beta = 0.04$, $P < 0.001$; ln(triglycerides): $\beta = -0.04$, $P = 0.02$), but not with BMI ($P = 0.4$), parameters of glucose metabolism (fasting and 2-hr glucose, fasting insulin, HOMA-IR, HbA1c; P between 0.1 and 1.0) and systolic or diastolic blood pressure ($P = 0.9$ and 0.09 , respectively). Associations between omentin and lipids were explained by adiponectin.

Conclusion: In conclusion, our data indicate that omentin is associated with lipid levels, but does not represent a novel biomarker of prediabetes and T2D. Supported by: DZD e.V., DFG (RA 459/3-1)

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The novel adipokine / hepatokine betatrophin is increased in women with gestational diabetes mellitus

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Background and aims: Betatrophin has recently been introduced as a novel adipokine/ hepatokine which promotes pancreatic β cell proliferation and improves glucose tolerance in mouse models of insulin resistance. However, regulation of betatrophin in women gestational diabetes mellitus (GDM), as well as its association with markers of obesity, glucose and lipid metabolism, inflammation, and renal function, have not been elucidated.

Materials and methods: Circulating betatrophin was quantified in 74 women with GDM and 74 healthy, pregnant, age-, body mass index-, and gestational age-matched controls by enzyme-linked immunosorbent assay. In a subset of the study population comprising of 85 patients (41 previous controls, 44 previous women with GDM), postpartum betatrophin levels were measured in a follow-up study.

Results: Median [interquartile range] serum betatrophin levels were higher in women with GDM (1.80 [0.53] $\mu\text{g/l}$) as compared to non-diabetic pregnant controls (1.58 [0.44] $\mu\text{g/l}$) ($p = 0.003$) during pregnancy. In multivariate analysis, GDM status was an independent and positive predictor of circulating betatrophin ($p < 0.05$). Furthermore, high density lipoprotein cholesterol and leptin were independently and positively associated with betatrophin (p

< 0.05). Moreover, betatrophin levels were significantly higher during gestation (1.69 [0.53] $\mu\text{g/l}$) as compared to postpartum levels (1.55 [0.66] $\mu\text{g/l}$) ($p = 0.029$).

Conclusion: Circulating betatrophin is increased in women with GDM and remains a positive predictor of GDM status. Betatrophin levels during gestation are significantly higher as compared to postpartum concentrations. Further studies need to elucidate the pathophysiological significance of betatrophin upregulation in GDM and during pregnancy.

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Plasma chemerin is a strong and independent predictor of cardiovascular event risk

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Background and aims: Associations of the adipokine chemerin with the metabolic syndrome (MetS) and with chronic kidney disease (CKD), two important indicators of increased cardiovascular event risk, have been described. However, the power of chemerin to predict cardiovascular events has not been investigated so far and is addressed in the present study.

Materials and methods: We measured plasma chemerin in a high-risk cohort of 495 patients undergoing coronary angiography for the evaluation of suspected or established coronary artery disease (CAD) in which cardiovascular events were prospectively recorded over 3.5 ± 1.1 years. Significant baseline CAD was diagnosed in the presence of coronary artery stenoses $\geq 50\%$.

Results: At baseline, plasma chemerin was significantly higher in patients with the MetS as defined by the current harmonized consensus definition ($n = 147$) than in non-MetS subjects (201 ± 71 ng/ml vs. 163 ± 62 ng/ml $p < 0.001$) and was inversely correlated with estimated glomerular filtration rate (eGFR; $r = -0.33$, $p < 0.001$). During follow-up, chemerin significantly predicted cardiovascular events ($n = 82$) univariately, after adjustment for age, gender, BMI, and eGFR, and also after additional adjustment for the presence of significant baseline CAD, with standardized hazard ratios of 1.83 [1.19 – 2.83], $p = 0.006$; 1.77 [1.12 – 2.80], $p = 0.015$; and 1.69 [1.07 – 2.67], $p = 0.024$, respectively.

Conclusion: From this first prospective evaluation of the cardiovascular event risk associated with chemerin we conclude that chemerin is strongly predictive of cardiovascular events independently from standard risk factors, from the MetS, and from the baseline presence of CAD.

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Secreted frizzled-related protein 4 is up-regulated in type 2 diabetes and interferes with hepatic insulin signalling

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Background and aims: Accumulating evidence implicates secreted regulators of the Wnt signaling pathway to metabolic control. For example, the inflammation-induced up-regulation of the Wnt inhibitor secreted frizzled-related protein 4 (Sfrp4) associates with impaired glucose-induced insulin secretion in type 2 diabetes. Importantly, Sfrp4 is also expressed in adipose tissue. The current study investigated whether the expression of Sfrp4 in visceral adipose tissue is altered in morbid obesity and type 2 diabetes, and whether Sfrp4 affects the expression of key rate-limiting gluconeogenic enzymes.

Materials and methods: Gene expression levels of Sfrp4 were determined by real-time PCR in subcutaneous and visceral adipose tissue biopsies from morbidly obese men ($n = 45$, of which 20 had type 2 diabetes) and control men ($n = 22$) undergoing abdominal surgery. The effects of recombinant Sfrp4 (100 ng/ml) on the mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6P) and fructose-1,6-bisphosphatase 1 (FBP1) were studied in the human hepatocyte cell line HepaRG after glucose starvation in the presence and absence of 100 nM insulin.

Results: In visceral adipose tissue, expression of Sfrp4 was 2.3-fold higher in patients with type 2 diabetes either versus patients with morbid obesity only or controls ($P<0.002$). There were no differences in Sfrp4 expression among these groups in subcutaneous adipose tissue. In vitro, in the absence of insulin, Sfrp4 reduced FBP1 expression by 33% ($P<0.05$), while having no effect on G6P- and PEPCK-levels in HepaRG cells. Insulin exposure reduced the expression of PEPCK, G6P and FBP1 by 2.1-, 2.8-, and 2.7-fold, respectively (all $P<0.001$). Exposing HepaRG cells to Sfrp4 further impaired the suppression of FBP1- and PEPCK-levels by insulin by 30% and 35%, respectively. In contrast, Sfrp4 did not affect the suppression of G6P levels by insulin. However, Sfrp4 reduced the insulin-mediated phosphorylation of Akt- and FOXO1, which has been implicated in the suppression of PEPCK expression, by 25% and 35%, respectively (both $P<0.05$).

Conclusion: These data show that the expression of the Wnt regulator Sfrp4 is selectively increased in visceral adipose tissue from patients with type 2 diabetes. In vitro, Sfrp4 interferes with the suppression of the expression of rate-limiting regulators of hepatic gluconeogenesis by insulin.

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Wnt1 inducible signalling pathway protein 1 (WISP1) is a novel marker of obesity regulated by high fat diet

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Background and aims: The Wnt signaling is involved in several processes such as proliferation, adhesion, apoptosis and differentiation. WISP1 (WNT1 inducible signaling pathway protein 1) is a target protein of canonical Wnt signaling and it belongs to the secreted extracellular matrix associated proteins of the CCN family. Recently, we showed that WISP1 expression is associated with signs of insulin resistance and low-grade inflammation in humans. We tested here the acute effect of insulin on WISP1 expression in vivo in subcutaneous adipose tissue (SAT). Additionally, the effect of high fat diet induced obesity on regulation of WISP1 gene expression in mice.

Materials and methods: Body weight matched, twelve-week-old male C57Bl/6J mice were kept on either a control diet D12450B (1) containing 10kcal-%fat, 20kcal-% protein, 70 kcal-% carbohydrate, 3.85kcal/g or a high fat diet D12492; (2) containing 60kcal-% fat, 20 kcal-% protein, 20 kcal-%carbohydrate, 5.24 kcal/g for 6 weeks. Body weight and composition was determined by magnetic resonance spectroscopy in conscious mice. Overnight fasted mice were sacrificed and epididymal white adipose tissue, liver and gastrocnemius muscle tissue were collected. Human mesenchymal stem cells (n=3) were differentiated in vitro into adipocytes. Differentiated adipocytes were incubated with 100nM insulin for 4h. Healthy, moderately obese male subjects (n=14) underwent one or three of the following procedures: 1) isotonic saline-infusion (n=11); 2) hyperinsulinemic-euglycemic clamp (EC) for 4 h with continuous infusion of 40•mU•m2 of the body surface•min-1 human insulin at a steady state capillary plasma glucose concentration of 4,4 mmol/l (80 mg/dl) (n=10); 3) hyperinsulinemic-hyperglycemic clamp (HC) for 4 h with continuous infusion of 40•mU•m2 of the body surface•min-1 human insulin at a steady state capillary plasma glucose concentration of 7,8 mmol/l (140 mg/dl) (n=8). Healthy, obese male subjects (n=10) underwent 1) isotonic saline-infusion and 2) intralipid/heparin -infusion. SAT biopsies were obtained before and after infusion. WISP1 gene expression was measured in human differentiated adipocytes, human SAT, in mice in epididymal white adipose tissue, in liver and gastrocnemius muscle tissue.

Results: WISP1 gene expression increased in human differentiated adipocytes after stimulation with 100nM insulin, but no significant difference was observed in SAT after HC and EC. The high fat diet-fed mice had increased body weight, fat mass and lean mass compared with control group and up-regulated WISP1 expression in adipose and muscle tissues. We couldn't observe any acute effect on WISP1 expression in human SAT after intralipid/heparin -infusion.

Conclusion: Insulin increased WISP1 expression in vitro, but has no acute effect in overweight subjects in vivo. High-fat diet induced obesity increased WISP1 gene expression in mice, however intralipid infusion hasn't any acute effect in overweight subjects in vivo.

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Wnt1 inducible signalling protein 1 (WISP1) is a novel adipokine linked to inflammation and insulin resistance in adipose tissue

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Background and aims: WISP1 (Wnt1 inducible signaling pathway protein 1) belongs to the secreted extracellular matrix associated proteins of the CCN family and is a target gene of canonical Wnt signaling pathway. Here we show that WISP1 is released from adipocytes and circulating WISP1 levels correlate with weight reduction and signs of insulin resistance.

Materials and methods: The mRNA expression of WISP1 was studied 1) in paired samples of visceral (VAT) and subcutaneous adipose tissue (SAT) from healthy glucose-tolerant subjects (n=75); 2) in paired VAT, SAT and liver tissue samples from subjects with/without NAFLD (n=47); 3) in overweight subjects underwent weight reduction by caloric restriction (n=49); 4) in SAT of overweight subjects (n=14) in the euglycemic-hyperinsulinemic (EC) and hyperglycemic-hyperinsulinemic (HC) clamp test; 5) in human mesenchymal stem cells (MSC) derived adipocytes and in human GM-macrophages. WISP1 protein levels were measured in overweight subjects underwent weight reduction and in cell culture medium adipocytes during differentiation. Expression and release of TNF α , IL-6, IL-10, IL-1 β was measured in differentiated adipocytes and in GM-macrophages after stimulation with 0,5 μ g/ml WISP1 for 24h. 3T3-L1 differentiated adipocytes were treated with 100nmol/L insulin with/without LY294002 (25 μ M, preincubation 30 min) and with/without PD 098059 (30 μ M, preincubation 30 min) for 4h.

Results: WISP1 protein release and WISP1 expression increase in cell culture medium during adipocytes differentiation. Expression and release of IL-6, IL-10, IL-1 β , TNF α in GM-makrophage was significantly enhanced after stimulation with 0,5 μ g/ml WISP1 for 24h. WISP1 expression was increased in differentiated 3T3-L1 cells after stimulation with 100nM insulin for 4h. This effect was abolished by incubation with LY294002 and PD098059. In human studies: 1) we found a correlation between insulin sensitivity, macrophages infiltration, adiponectin levels and WISP1 gene expression in samples of VAT and SAT; 2) WISP1 expression is higher in (VAT) rather than in (SAT); 3) hepatic WISP1 expression has no association with ectopic fat accumulation in obesity; 4) weight reduction decreases WISP1 expression in SAT as well as circulating WISP1 levels in plasma.

Conclusion: Our data shows that WISP1 is a novel adipokine but no hepatokine that is substantially overexpressed in visceral fat from obese subjects and reflects insulin resistance and adipose tissue inflammation. Weight changes regulate circulating WISP1 levels and WISP1 expression in adipose tissue. Therefore, we propose that WISP1 is a novel biomarker and a potential link between obesity and the metabolic syndrome.

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FABP5 is an essential modulator of fatty acid-induced GIP secretion in enteroendocrine K cells

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Background and aims: Fatty acid-binding protein 5 (FABP5) is a 15-kDa cytosolic-protein with high affinity to long chain fatty acids (LCFA). It has been known as an intracellular chaperon that transports LCFA into various organelles and has been reported to be involved in insulin resistance in white adipose tissue (WAT). Using microarray analysis, we demonstrated that FABP5 is expressed exclusively in murine enteroendocrine GIP-producing K-cells. We investigated the physiological function of FABP5 in K-cells.

Materials and methods: Immunostaining for FABP5 and GIP was performed in murine samples of small intestine for confirmation of specific expression of FABP5 in K-cells. To evaluate the acute GIP secretory response in FABP5^{-/-} mice, lard (10ml/kg) and glucose were injected orally; plasma glucose and serum levels of GIP, GLP-1 and insulin were measured. Using K-cells isolated from newly-generated GIP-GFP knock-in hetero (GIPgfp/+)-FABP5^{+/+} and GIPgfp/+ -FABP5^{-/-} mice, quantitative real-time PCR (qPCR) for GIP expression and measurement of GIP content were performed to determine whether or not FABP5 is involved in GIP biosynthesis. To assess potential effects of FABP5-deficiency on body weight and composition, mice were fed a high fat diet (HFD) for 10 weeks. Whole body CT scans of HFD-fed wild-type (WT), FABP5^{-/-}, GIPgfp/gfp-FABP5^{+/+} and GIPgfp/gfp-FABP5^{-/-} mice were compared. Using WAT taken from HFD-fed WT and FABP5^{-/-} mice, mRNA expression levels of lipid metabolism-related genes were evaluated by qPCR.

Results: Immunostaining of intestinal mucosa showed that GIP-positive cells were totally merged with FABP5-positive cells and that 90% of FABP5-positive cells were merged with GIP-positive cells. Although plasma GIP levels after lard injection were significantly lower in FABP5^{-/-} mice compared to those in WT mice, there were no significant differences in the results of OGTT. Plasma glucose, insulin and GLP-1 levels after both glucose and lard administration were similar in WT mice and FABP5^{-/-} mice. There was no significant difference in GIP mRNA expression or GIP content in K-cells from GIPgfp/+ -FABP5^{+/+} and GIPgfp/+ -FABP5^{-/-} mice. Under HFD feeding conditions, FABP5^{-/-} mice exhibited significantly decreased body weight gain compared to WT control, but there was no significant difference in body weight between GIPgfp/gfp-FABP5^{+/+} and GIPgfp/gfp -FABP5^{-/-} mice, in which GIP expression is genetically deleted. Whole body CT scan showed that body fat mass was significantly reduced in FABP5^{-/-} mice compared to that in WT mice and that body fat mass in GIPgfp/gfp -FABP5^{+/+} and GIPgfp/gfp -FABP5^{-/-} mice was comparable. qPCR of WAT revealed no significant change in mRNA expression levels of hormone sensitive lipase, PPAR δ , aP2 or GIP-receptor between HFD-fed WT and FABP5^{-/-} mice.

Conclusion: Our results show that FABP5 is involved in fatty acid-induced acute GIP secretion and that it contributes to the development of HFD-induced obesity in a GIP-dependent manner.

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NOV/CCN3: a new adipokine involved in obesity-associated insulin resistance?

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Background and aims: The pathophysiology of obesity is far to be elucidated. Identification of new adipokines represents a major challenge to decipher the mechanisms of adipose tissue development and metabolism. Our recent results suggest that NOV/CCN3, a multifunctional matricellular protein, is synthesized and secreted by adipose tissue, with plasma levels correlated with body mass index and fat mass, and down-regulated following bariatric surgery (Pakradouni et al.). NOV has been involved previously in tissue repair, fibrotic and inflammatory diseases, and cancer. However, its role in adipose tissue and energy homeostasis remains unknown.

Materials and methods: The aim of our work was to investigate the metabolic phenotype of NOV knockout mice fed a standard or high fat diet (HFD) **Results:** Weight gain was similar between wild type (WT) and NOV^{-/-} mice fed a chow diet. Strikingly, the weight of NOV^{-/-} mice fed a HFD was markedly lower than that of WT animals. This was related to a sharp decrease in fat mass, whereas lean mass was preserved. Accordingly, NOV^{-/-} mice fed a HFD displayed an improved glucose tolerance and insulin sensitivity. No significant changes were detected for food intake, energy expenditure, or locomotor activity. Importantly, the absence of NOV was associated with a marked decrease in adipose tissue expression of several proinflammatory cytokines and chemokines. Conversely, exposure of 3T3-L1 mature adipocytes to NOV led to an induction of these cyto-/chemokines.

Conclusion: Altogether, these results show that NOV is a new adipokine that could be involved in obesity-associated insulin resistance

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The mRNA expression and release of (pro)-inflammatory and angiogenic factors in perivascular fat cells is influenced by fetuin-A via different signalling pathways

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Background and aims: Our previous in vitro and in vivo studies showed that fetuin-A exerts effects on perivascular fat cells (PVFC) and vascular cells which may influence cardiovascular diseases, predominantly effects on (pro)- inflammatory and angiogenic factors. Analysis of underlying pathways demonstrated that fetuin-A/palmitate (fet/palm) stimulates IL-6 and -8 expression via TLR4 receptor signalling. In contrast, the inhibition of the potent angiogenic protein HGF was mediated via the well known insulin-dependent receptor tyrosine kinase pathway. In order to elucidate pathways downstream TLR4 receptor activation, in this study fet/palm induced signalling pathways were studied in PVFC more detailed using several kinase inhibitors.

Materials and methods: PVFC were isolated from fat specimens around arm arteries of patients from our trauma center. Cells were treated with or without the JNK inhibitor SP500125 (10 μ mol/L), the p38MAPkinase inhibitor SB203580 (10 μ mol/L), the MEK1/2 inhibitor PD98059 (10 μ mol/L) and the NF κ B inhibitor BAY11-7082 (1 μ mol/L) 2 h prior to the addition of fet (600 μ mol/L) and palm (50 μ mol/L). After 24 h protein release was quantitated by ELISA technology and mRNA expression by realtime PCR.

Results: The expression of IL-8, IL-6 and MCP-1 was upregulated by fet and this effect was potentiated significantly by the addition of palm. Additional incubation with the NF κ B inhibitor abrogated these effects significantly. JNK inhibition caused a partial blockade of the fet/palm induced stimulatory effects on interleukins whereas MEK1/2 and p38MAPkinase inhibition had only slight influence on the potent stimulatory effects of fet/palm. In contrast to the TLR4-mediated stimulatory effects on interleukins, the expression of the strong angiogenic factor HGF was potentially inhibited by fet/palm and this effect could not be abrogated by the NF κ B inhibitor and only partially by the other MAPkinase inhibitors. However, the angiogenic factor bFGF was not inhibited but slightly stimulated by fet/palm and this was predominantly influenced by the JNK inhibitor and slightly by NF κ B inhibitor. Finally, fet/palm exerted nearly no effects on the angiogenic factor VEGF.

Conclusion: Fet/palm caused strong stimulatory effects on (pro)inflammatory proteins. However, the observed effects on several potent angiogenic factors were diverse: bFGF was stimulated slightly, VEGF remained nearly unchanged and HGF was even inhibited significantly. Treatment with several signalling pathway inhibitors showed that fet acts via different pathways downstream TLR4 receptor activation, such as JNK or NF κ B signalling but to a lesser extent on other MAPkinases, such as MEK1/2 and p38. Nevertheless, the diverse inhibitory effect of fet/palm on the potent angiogenic factor HGF seems to be mediated at least partially by another - TLR4-independent - pathway.

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NAMPT knockdown attenuates atherosclerosis by promoting macrophage reverse cholesterol transport

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Background and aims: Nicotinamide phosphoribosyltransferase (NAMPT), also known as visfatin, was claimed to be secreted predominantly from visceral adipose tissue and to exert insulin-like action. It has been reported that NAMPT induced the release of inflammatory cytokines, cholesterol accumulation and the formation of macrophage foam cells in cultured cells. So there may be some association with atherosclerosis due to its role in inflammation and lipid accumulation. However, the impact of NAMPT on atherosclerosis

remained to be systematically investigated. To determine the role of NAMPT in the development of atherosclerosis, we have determined whether NAMPT knockdown can improve insulin resistance and formation of atherosclerotic lesions by using a loss-of-function approach (adenovirus-mediated RNAi) to inhibit NAMPT expression in the liver in C57BL/6J and apoE KO mice on a high fat diet. More importantly, we discussed the molecular mechanisms of NAMPT-knockdown in the regulation of cholesterol metabolism and the development of atherosclerosis.

Materials and methods: In vivo, NAMPT-knockdown C57BL/6J and apoE KO mice by a adenovirus-mediated RNAi was used to assessed the effects of NAMPT knockdown on metabolic parameters on a standard chow diet or a high fat diet; Euglycemic- hyperinsulinemic clamps were performed to assess glucose kinetics by tracer dilution methodology. An in vivo reverse cholesterol transport (RCT) assay that traces ^3H -cholesterol derived from macrophages loaded with cholesterol ex vivo was performed. In vitro, assays testing effects of Ad-GFP or Ad-*sh*Visfatin treatment on cholesterol efflux from ^3H -cholesterol-loaded RAW264.7 cells to lipoprotein acceptors was conducted. The expressions of hepatic genes related to cholesterol metabolism were analyzed by qRT-PCR and western blots; MK886, a selective inhibitor of PPAR α was used to analyze the mechanisms by which NAMPT knockdown leads to increased cholesterol efflux and RCT.

Results: HFD-fed C57BL/6J mice with reduced hepatic NAMPT content showed no alterations in all tested metabolic parameters, except for elevated HDL-C and slightly decreased LDL-C levels without statistical significance compared to littermates fed HFD; However, plasma HDL-C levels in HFD-fed ApoE KO mice treated with Ad-*sh*NAMPT were significantly increased and hepatic TC contents were decreased compared to littermates fed HFD; Analysis of lipoproteins by FPLC showed an increase in cholesterol content in the HDL fractions of Ad-*sh*NAMPT-treated mice compared with control mice; RCT assay showed that higher HDL levels in response to NAMPT knockdown increased cholesterol transport from peripheral macrophages to the liver. In cultured RAW264.7 cells, HDL-mediated ^3H -cholesterol efflux was increased by NAMPT knockdown; NAMPT knockdown upregulated mRNA and protein expression involved in cholesterol efflux and RCT; MK886 abolished NAMPT knockdown-induced ABCA1/G1 and LXR α expressions in Hepa1-6 and RAW264.7 cells.

Conclusion: NAMPT knockdown exerted antiatherogenic effects by promoting cholesterol efflux and macrophage RCT through the PPAR α - LXR α -ABCA1/G1 pathway in vitro or in vivo.

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Dietary fat intake in low-carbohydrate diets and subsequent mortality and weight change in type 2 diabetes

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Background and aims: The replacement of carbohydrates with fat in a diet for type 2 diabetes patients is still debated. This study aimed to investigate the association between dietary carbohydrate intake and replacement with different types of fat with all-cause and cardiovascular (CVD) mortality risk and 5-year weight change in patients with type 2 diabetes.

Materials and methods: The study included 6,192 patients with type 2 diabetes from 15 cohorts of the European Prospective Investigation into Cancer and Nutrition (EPIC). Dietary intake was assessed at baseline with country-specific food-frequency questionnaires. Cox regression was used to estimate whether replacement of carbohydrates with different types of fat was associated with cardiovascular mortality adjusted for diabetes risk factors and dietary factors. Linear regression was used for associations with weight change.

Results: After a mean follow-up of 9.2 ± 2.3 y, 791 (13%) participants had died, of which 268 (4%) due to CVD. The risk of all-cause mortality increased when 10 gram or 5 energy % of carbohydrates was replaced by total fat (HR 1.07 [1.02-1.13]), or SFAs (HR 1.25 [1.11-1.40]) and decreased when replaced by MUFAs (HR 0.89 [0.77-1.02]). CVD mortality risk increased when carbohydrates were substituted with SFAs (HR 1.22 [1.00-1.49]) or PUFAs (HR 1.29 [1.02-1.63]). The 5-year weight change decreased when carbohydrates were substituted with total fat or MUFAs, and increased when substituted by SFAs. After adjusting for 5-year weight change, the associations for (CVD) mortality lost significance.

Conclusion: In diabetes patients, substitution of carbohydrates with total fat or SFAs increased mortality risk. Replacement of carbohydrates with MUFAs was associated with lower mortality risk and weight change. Dietary guidelines should focus on replacement of carbohydrates by fat-subtypes, especially replacement of SFAs by MUFAs seems beneficial.

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Differential acute postprandial effects to isocaloric meals with different macronutrient content in subjects with type 2 diabetes and healthy controls

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Background and aims: Dietary guidelines for treatment of diabetes focus mainly on carbohydrate counting. Evidence suggests that postprandial metabolic response is also modified by other macronutrient content of meals and their effect on various gastrointestinal hormone release. We aimed to investigate the effects of two standardized isocaloric meals: a processed hamburger meat meal rich in protein and saturated fat (M-meal) and a vegetarian sandwich meal rich in carbohydrates (V-meal) in terms of postprandial glucose, lipids and insulin levels, gastrointestinal hormone (GIH) response and oxidative stress markers.

Materials and methods: In a randomized crossover study, 50 patients with type 2 diabetes (T2D) and 50 healthy subjects underwent two 3-hour meal tolerance tests. For statistical analyses, repeated-measures ANOVA was used.

Results: The M-meal resulted in a higher postprandial increase in lipids in both groups ($p < 0.001$) and persistent postprandial hyperinsulinemia in diabetics. The plasma glucose levels were significantly higher after the V-meal only at the peak level. The GIH concentrations were higher (glucose-dependent insulinotropic peptide (GIP): $p < 0.001$; peptide tyrosine-tyrosine (PYY): $p < 0.05$; pancreatic polypeptide (PP): $p < 0.01$) and the ghrelin concentration

was lower ($p < 0.05$) after the M-meal in healthy subjects. In contrast, the GIH concentrations were significantly higher after the V-meal in T2D patients (glucagon-like peptide-1: $p < 0.001$; GIP: $p < 0.001$; PYY: $p < 0.05$; PP: $p < 0.05$). Compared with the V-meal, the M-meal was associated with a larger increase in lipoperoxidation in T2D patients (thiobarbituric acid reactive substances: $p < 0.05$).

Conclusion: Our results suggest that the diet composition and the energy content, rather than the carbohydrate count, should be important considerations for dietary management and demonstrate that differences in isocaloric meals composition led to differences in postprandial gastrointestinal hormone response and oxidative stress.

Clinical Trial Registration Number: NCT01572402

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Dietary protein intake in low-carb diets and subsequent weight change and mortality in type 2 diabetes

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Background and aims: Dietary replacement of carbohydrates with protein for patients with type 2 diabetes is still debated. This study aimed to investigate the association between dietary carbohydrate intake and replacement with (animal and plant) protein, with 5-year weight change and all-cause and cardiovascular (CVD) mortality risk in patients with type 2 diabetes.

Materials and methods: The study included 6,192 patients with type 2 diabetes from 15 cohorts of the European Prospective Investigation into Cancer and Nutrition (EPIC). Usual dietary intake was assessed at baseline with country-specific food-frequency questionnaires. Different methods to adjust for energy intake were used.

Results: Annual weight-loss of participants was 0.17 (SD 1.24) kg. After a mean follow-up of 9.2 (SD 2.3) y, 791 (13%) participants had died, of which 268 (4%) due to CVD. Substitution of dietary carbohydrate intake with plant protein was associated with reduced risk of all-cause mortality (HR 0.71 [0.61–0.89]) and CVD mortality (HR 0.68 [0.53–0.89]). Substitution with total protein or animal protein was not associated with total or CVD mortality risk. Five-year weight change increased by substitution with total protein (β 151 [39–262] g) and animal protein (β 156 [39–271] g), but not by substitution with plant protein (β 279 [–102–660] g). Five-year waist circumference change was not associated with any substitution of carbohydrates with protein. After adjustment for 5-year weight change, the association with CVD mortality was attenuated and became non-significant, but remained the same for all-cause mortality.

Conclusion: In diabetes patients, replacement of dietary carbohydrates with total protein and animal protein was associated with weight gain, but not with total and CVD mortality risk. Substitution of carbohydrates with plant protein may reduce the risk of total and CVD mortality.

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Does excessive consumption of high fructose corn syrup, aspartame or rebaudioside A affect insulin sensitivity and regulatory genes in liver and muscles?

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Background and aims: Added sugar is believed to play a critical role in the epidemic of welfare diseases and in particular, fructose, has been shown to be directly diabetogenic. Furthermore, intake of the added sweetener high-fructose corn syrup (HFCS), consistently correlates with increased risk for developing lifestyle diseases. The little information regarding non-caloric sweeteners such as Rebaudioside A and Aspartame. The aim of this study is to investigate the effects of long-term high-fructose (HF) feeding on Development of insulin resistance (IR), steatosis and dyslipidaemia in rats; and to

examine the effects of intervention sweetener diets post the HF feeding e.g. excessive daily intake of HFCS, Rebaudioside A and Aspartame.

Materials and methods: 48 healthy male Wistar rats were randomly assigned to four groups. One control group and three intervention groups received a diet of 60% fructose for 16 weeks. Post HF feeding the three groups instead received water sweetened with HFCS, Rebaudioside A or Aspartame for 8 weeks. Development and progression of IR, steatosis, dyslipidaemia and hypertension was monitored and tissues were analyzed after termination.

Results: The fructose diet led to a significant increase in insulin resistance, fasting levels of TG, total- and LDL-cholesterol. No differences in weight were observed. When compared with control group the daily intake of HFCS resulted in large increases in fasting blood TG levels (0.71 ± 0.04 vs 1.53 ± 0.14 mM; $p < 0.005$); Total cholesterol (1.45 ± 0.05 vs 1.87 ± 0.12 mM; $p < 0.05$); development of IR HOMA-IR: (55.55 ± 10.69 vs 28.20 ± 21.67 ; $p < 0.01$) and a 43 % increased storage of lipids in liver and muscle tissue (MR-scanning). Furthermore, HFCS markedly changes the gene expression profile of key regulatory genes as Srebp-1, FAS in the liver and FAS and GLUT 4 as well as PGC1A in muscle tissue. Rebaudioside A or Aspartame did not change any of the above shown data significantly when compared with control group.

Conclusion: HF feeding and excessive daily intake of HFCS had a negative physiological impact in this study. Switching to Rebaudioside A or aspartame reversed the hypercholesterolaemia induced fructose feeding and improved insulin sensitivity. Excessive HFCS-intake caused IR within 8 weeks and increased lipid storage in liver and muscle tissue.

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A low-dose whey protein preload slows gastric emptying and improves post-prandial glycaemia in type 2 diabetes

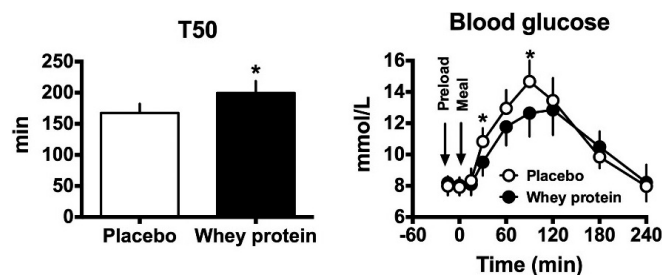
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Background and aims: We previously reported that a 55g whey protein 'preload', taken 30 minutes before a meal, reduced postprandial glycaemia in patients with type 2 diabetes, related in part to slowing of gastric emptying. A smaller preload that could be taken a lesser interval before the meal would be advantageous in terms of cost, reduction in additional energy intake, and compliance. We therefore evaluated the effects of a low-dose 20g whey protein preload, formulated with 5g guar, on post-prandial glycaemia and gastric emptying.

Materials and methods: Seven subjects with type 2 diabetes [4 males and 3 females; age 64.6 ± 0.4 years; body mass index: 29.3 ± 2.0 kg/m²; HbA1c $6.8 \pm 0.4\%$ (50.4 ± 3.9 mmol/mol); duration of known diabetes 4.8 ± 0.8 years], managed by diet or metformin only, were each studied on 2 occasions in randomised order. On each day, a 150 mL preload drink containing either 20 g whey plus 5 g guar, or similarly flavoured placebo, was consumed 15 minutes before a 13C-octanoic acid-labelled mashed potato meal. Gastric emptying (breath test) and blood glucose (glucometer) were evaluated. Data are means \pm SEM.

Results: The half-emptying time (T50) of the potato meal was greater (199.7 ± 20.8 vs. 167.4 ± 16.0 min, $P = 0.004$) after the whey protein preload than placebo. Fasting blood glucose did not differ between the two study days, and was not altered by either preload prior to the meal ($t = -15$ to 0 min). After the meal ($t = 0$ to 240 min), blood glucose concentrations were lower after whey protein than placebo ($P < 0.001$ for treatment \times time interaction, with significant differences at $t = 30$ and 90 min after the meal, $P < 0.05$ for each).

Conclusion: A low-dose whey protein preload can slow gastric emptying and improve postprandial glycaemic excursions in patients with type 2 diabetes who have relatively good glycaemic control. It may represent a promising nutritional strategy to optimise postprandial glycaemia in this group.



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Two weeks of adaptation to a high-protein hypercaloric high-fat-diet changes the postprandial carbohydrate and fat response after a mixed mealA. Rietman¹, J. Schwarz¹, E. Siebelink¹, F.J. Kok¹, D. Tomé², M. Mensink¹;¹Human Nutrition, Wageningen University, Netherlands,²Nutrition Physiology and Ingestive Behavior, AgroParisTech, INRA, UMR914, Paris, France.

Background and aims: We and others previously showed changes in fasting lipid metabolism after adaptation to a high-protein diet. To further explore these changes we evaluated the effect of a two week short-term adaptation to a high protein diet on postprandial carbohydrate and lipid metabolism in healthy human subjects, by applying a meal challenge (MC).

Materials and methods: A randomized crossover trial with a parallel control group was performed. After a 2-week-run-in period, participants were assigned to either the control-diet for 4 weeks (CD-group; n=10; 27.8 En% fat; 16.9 En% protein; 55.3 En% carbohydrates), or a hypercaloric high-fat diet (HD-group; n=17; + 2 MJ per day), with in random order two periods of 2 weeks, with either a high protein (HP; 37.7 En% fat; 25.7 En% protein; 36.6 En% carbohydrates) or a normal protein content (NP; 39.4 En% fat; 15.4 En% protein; 45.2 En% carbohydrates). The MC (Liquid meal, 40 En% fat; 15 En% protein and 45 En% carbohydrate) was performed after 2 weeks and 4 weeks of intervention. Blood samples were drawn regularly during 6 hours postprandial for metabolite analyses.

Results: No significant differences were observed between NP and HP in fasting glucose (5.04 ± 0.08 vs. 5.05 ± 0.09 mmol/L) and insulin (4.21 ± 0.62 vs. 3.95 ± 0.63 μ U/L) levels. However, during the MC, subjects who were adapted to the HP diet showed a significantly higher iAUC of glucose as compared to subjects adapted to the NP diet (NP: 377.4 ± 14.1 vs. HP: 408.2 ± 13.2 mmol/L*60min; $p=0.03$), without clear differences in insulin iAUC (NP: 2583.9 ± 383.7 vs. HP: 2984.8 ± 496.9 mU/L*60 min; $p=0.38$). Fasting triglyceride (TG) concentration tended to be lower on the HP compared to the NP condition (NP: 0.77 ± 0.05 vs. HP: 0.65 ± 0.03 mmol/L; $p=0.07$). Also during the MC subjects adapted to the HP diet showed a lower iAUC of TG as compared to subjects adapted to the NP diet (NP 67.1 ± 5.6 vs. HP 57.9 ± 4.4 mmol/L*60min; $p=0.11$).

Conclusion: Adaptation to a high protein hypercaloric high-fat diet resulted in a decreased clearance of carbohydrates after the MC as indicated by the increased glucose iAUC, without changes in plasma insulin response compared to adaptation to a normal protein hypercaloric high-fat diet. Additionally, adaptation to the HP diet was associated with an improved lipid metabolism, as indicated by a lower level of circulating TG in fasted state as well as during the MC. Additional metabolomics data are anticipated to be present at the time of the meeting.

Clinical Trial Registration Number: NCT01354626

Supported by: NZO

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Differential islet, incretin and glucose prandial responses after high caloric breakfast vs isocaloric dinner in type 2 diabetic patientsD. Jakubowicz¹, O. Froy², Y. Bar-Dayana¹, H. Rabinovitz², Z. Landau¹, J. Wainstein¹;¹Diabetes Unit, E. Wolfson Medical Center, Holon,²Institute of Biochemistry, Food Science and Nutrition, Rehovot, Israel.

Background and aims: It was shown in type 2 diabetic (T2D) individuals that a high-calorie (HC) that a diet with high-calorie (HC) breakfast (B) with reduced dinner (D), resulted in a significant decrease in HbA1c, and was more beneficial than isocaloric diet with HCD and reduced B in improving glucose, lipids and hunger scores. Whether it is related to a potential diurnal pattern of incretin secretion and β -cell responsiveness to incretins after Iso HCB vs HCD has however, not been studied in T2D. We assessed in T2D postprandial early AUC30, late AUC 60-180 and AUC180 min response for plasma insulin, glucose, intact (i) and total (t) glucagon-like peptide-1 (GLP-1) and hunger scores after HCB vs isocaloric HCD

Materials and methods: In a randomized crossover design 18-T2D (8 males), aged 57 ± 83 yrs; BMI: 28.11 ± 2.92 kg/m²; HbA1c: $7.55 \pm 0.42\%$, undergone HCB meal test on day with HCB diet: B: 700 kCal; % of CH: protein: fat: 50:30:20%; lunch (L) (500 kCal, 20:45:25%) and D (200 kCal, 13:40:47%). Another day HCD meal test was done on HCD diet: B: (200 kCal, 13:40:47%), L: (500 kCal, 20:45: 25%) and D (700 kCal, 50:30:20%).

Results: Glucose peak was lower after HCB: 230 ± 24 vs. HCD: 285 ± 25 mg/dl, ($p<0.001$). Similarly, AUC180 for glucose was reduced after HCB: 31255 ± 1375 vs HCD: 40899 ± 3007 mg/dl*min, ($p<0.001$). AUC 60-180 for late insulin secretion was not different ($p<0.11$), in contrast AUC30 for early insulin response was significantly greater (almost 2-fold) after HCB: 526 ± 109 vs. HCD: 247 ± 47 mIU/ml*min, ($p<0.001$). Postprandial AUC180 response after HCB vs after HCD was higher by $29 \pm 9.7\%$ for tGLP-1 ($p<0.001$) and by $20.7 \pm 7\%$ for iGLP-1 ($p<0.001$) and reduced by $44.2 \pm 11\%$ for hunger scores ($p<0.001$). AUC30 for tGLP-1 and iGLP-1 were strongly and positively associated with AUC30 early insulin response ($r=0.32$, $p<0.0001$); whereas negatively associated with the postprandial AUC180 for plasma glucose ($r=0.18$, $p<0.0001$).

Conclusion: HCB induced more rapid early (30-min) insulin response associated with greater total and intact GLP-1, lower glucose excursion and greater hunger suppression. It suggests that high caloric intake in the breakfast with reduced intake at dinner might be a beneficial alternative for the management of type 2 diabetes.

Clinical Trial Registration Number: NCT01977833

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The effects of a high egg diet in people with type 2 diabetes mellitus: a three-month randomised controlled trialN.R. Fuller¹, I.D. Caterson¹, G. Denyer^{2,1}, A. Sainsbury¹, T.P. Markovic¹;¹Sydney Medical School,²Faculty of Sciences, The University of Sydney, Australia.

Background and aims: The effects of high egg consumption in people with Type 2 Diabetes Mellitus (T2DM) are controversial, due to many confounding factors in previously published research. This randomised, controlled study aimed to determine whether a high egg diet (two eggs per day on six days per week) leads to improved lipid profiles (increased high density lipoprotein cholesterol; HDL-C) in people with pre-diabetes or T2DM, compared to a low egg diet (less than two eggs per week).

Materials and methods: 140 subjects with pre-diabetes or T2DM were randomised to either a low or high egg diet as part of a weight maintenance study. Subjects attended the clinic monthly and were given a written guide as to the specific types of foods and quantities that could be consumed, with particular emphasis on improving management of T2DM, and replacing foods containing saturated fats with foods containing monounsaturated fats and polyunsaturated fats. Outcome measures were collected at screening and three months.

Results: There was no significant difference in the change in HDL-C from screening to three months between the two groups; mean difference (95% confidence interval (CI)) for the high egg versus low egg group: $+0.02$ mmol/L (-0.03 , 0.08); $P=0.38$. There was a within group trend towards an improvement in HDL-C in the high egg group: $+0.034$ mmol/L (95% CI: -0.003 , 0.071); $P=0.07$. No between group differences were found at three months for total cholesterol, low density lipoprotein cholesterol, triglycerides, or glycaemic control. Both groups were matched for protein intake, yet the high egg group reported less hunger and greater satiety post meal. Both diets were well accepted, with the high egg group reporting a significantly greater enjoyment of foods, less boredom, and more satisfaction with the diet.

Conclusion: Eggs do not have an adverse effect on lipid levels in those with T2DM and hence at high risk of cardiovascular outcomes. Moreover, it is associated with better appetite control. These findings suggest that a high egg diet can be included as part of the dietary management of T2DM.

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Assessment of caffeine dose and time of administration required for resetting of insulin sensitivity in high-sucrose ratsJ.F. Sacramento¹, J.C. Coelho¹, B.F. Melo¹, M.P. Guarino^{1,2}, S.V. Conde¹;¹CEDOC, Faculdade Ciências Médicas, Universidade Nova de Lisboa,²UIS- Unidade de Investigação em Saúde- Escola Superior de Saúde de Leiria- Instituto Politécnico de Leiria, Portugal.

Background and aims: Several epidemiological studies have described beneficial effects of chronic coffee intake on type 2 diabetes and metabolic syndrome. Our group has previously shown that chronic caffeine intake (1g/L over 15 days) prevents the development of insulin resistance (IR) and hypertension in diet-induced IR rats. The aim of this work was to investigate the

therapeutic dose of caffeine that restores insulin sensitivity and normalizes blood pressure in diet-induced IR rats. The time required for the reversion of IR was also evaluated.

Materials and methods: All experiments were performed in 8–13 weeks Wistar rats of both genders (250–450 g). Two groups of rats were used: a high-sucrose diet-group (HSu) and a control group. The HSu insulin-resistant model was obtained by submitting the animals to a 35% sucrose diet during 28 days. A dose-response curve for chronic caffeine intake (0.25–1 g/L) on insulin sensitivity was performed both in HSu and control groups. Caffeine was administered in drinking water after the first 28 days of control or HSu diet and maintained during 13 weeks. The insulin sensitivity was evaluated by an insulin tolerance test in conscious animals. Plasma glucose and insulin were monitored each 2 weeks. At the end of the experimental period, rats were anaesthetized with pentobarbitone (60 mg/Kg) and blood pressure was measured in the femoral artery. Blood was collected by heart puncture and skeletal muscle collected for Glut4, Insulin receptor and AMPK α 1 quantification by Western-blot.

Results: Administration of HSu diet during 28 days decreased insulin sensitivity to 2.81 ± 0.29 %glucose/min (KITT control = 4.70 ± 0.28 %glucose/min). Chronic administration of 0.5 and 1 g/L of caffeine did not modify insulin sensitivity. In the HSu group, 3 weeks after chronic administration of 1 g/L of caffeine, insulin sensitivity was restored (KITT = 4.70 ± 0.299 %glucose/min). The insulin sensitivity in this group was maintained during the following weeks. In the HSu rats submitted to chronic administration of 0.5 g/L caffeine insulin sensitivity start to increase (30%) after 5 weeks of administration and it was completely restored after 9 weeks (KITT = 4.75 ± 0.669 %glucose/min). **Conclusion:** Chronic caffeine administration in all concentrations tested restores insulin sensitivity. The period of time to restore insulin sensitivity is positively related with caffeine concentration.

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A fish-based diet intervention improves endothelial function in postmenopausal women with type 2 diabetes mellitus: a lipidomics approach to understand the mechanisms

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Background and aims: The beneficial effects of fish and n-3 polyunsaturated fatty acids (PUFA) consumption on atherosclerosis have been reported in numerous epidemiological studies. However, to the best of our knowledge, the effects of a fish-based diet intervention on endothelial function have not been investigated. Therefore, we studied these effects in postmenopausal women with type 2 diabetes mellitus (T2DM).

Materials and methods: Twenty-three postmenopausal women with T2DM were assigned to two four-week periods of either a fish-based diet (n-3 PUFA 3.0 g/day) or a control diet in a randomized crossover design. Endothelial function was measured with reactive hyperemia using strain-gauge plethysmography and compared with the serum levels of fatty acids and their metabolites. Endothelial function was determined with peak forearm blood flow (Peak), duration of reactive hyperemia (Duration) and flow debt repayment (FDR). Serum lipid compositions and eicosanoids were measured with tandem mass spectrometry (LCMS).

Results: A fish-based dietary intervention improved Peak by 63.7%, Duration by 27.9% and FDR by 70.7%, compared to the control diet. Serum n-3 PUFA levels increased after the fish-based diet period and decreased after the control diet period, compared with the baseline period (1.49 vs. 0.97 vs. 1.19 mmol/l, $p < 0.0001$). There was no correlation between serum n-3 PUFA levels and endothelial function. Eicosanoids were measured with LCMS and 7 / 86 metabolites were significantly changed after a fish-based diet intervention. Interestingly, the ratio of epoxyeicosatrienoic acid (EET, known as the vasodilator) / dihydroxyeicosatrienoic acid (the inactive metabolites of EET) was increased after a fish-based diet intervention.

Conclusion: A fish-based dietary intervention improves endothelial function in postmenopausal women with T2DM. The improvement of endothelial function was not associated with serum n-3 PUFA concentration. These data suggests that the vasoactive metabolites such as EET, may contribute to this phenomenon.

Clinical Trial Registration Number: UMIN000002277

PS 058 Nutrition and diet II

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Incretin hormones are released in healthy subjects by both diet oil and olive oil while short chain fatty acids have no effect on secretion

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Background and aims: Diet oil (1,3-di-butyl-2-oleoyl-glycerol) consists of two short chain fatty acids and 2-oleoyl-glycerol (2-OG), the latter being a naturally occurring ligand for the G protein-coupled receptor GPR119. Intestinal 2-OG causes release of the incretin hormone, glucagon-like peptide-1 (GLP-1), in healthy subjects and diet oil does the same in patients with type 2 diabetes. This study evaluated tributyrin, which in mouse studies may increase GLP-1 release, and a new form of diet oil with medium chain fatty acids (1,3-di-octanoyl-2-oleoylglycerol or “C8-diet oil”) on GLP-1 and glucose-dependent insulintropic polypeptide (GIP) secretion. Octanoic acid may not significantly activate known fatty acid receptors.

Materials and methods: C8-diet oil, tributyrin, olive oil and carrot were administered orally to 12 Caucasian healthy male subjects (age: 24 ± 0.6 years (mean \pm SEM), body mass index: $22. \pm 0.6$ kg/m²; HbA1c: 30.45 ± 0.67 mmol/mol) in a randomised, single-blinded cross-over study. The subjects were given four meals on four different days: 200 g grated carrot + 6.53 g tributyrin; 200 g grated carrot + 13.15 g diet oil; 200 g grated carrot + 19 g olive oil or 200 g grated carrot. All lipids amounted to 0.0216 mol, and theoretically, the C8-diet oil and olive oil regimens both result in formation of 0.0216 mol 2-OG during digestion. Primary outcomes were total GLP-1 and GIP in plasma.

Results: C8-diet oil and olive oil resulted in ($p < 0.01$) greater postprandial GLP-1 and GIP responses [GLP-1: incremental area under curve 583 and 538 pmol/l \times 120 min, $p = 0.733$; GIP: 1674 and 3293 pmol/l \times 120 min, $p = 0.002$] compared to both tributyrin (GLP-1: 94 pmol/l \times 120 min and GIP: 403 pmol/l \times 120 min) and the carrot meal (GLP-1: 126 pmol/l \times 120 min and GIP: 423 pmol/l \times 120 min).

Conclusion: C8-diet-oil and olive oil enhanced secretion of GLP-1 and GIP significantly compared to tributyrin and carbohydrate. Tributyrin had no effect. Olive oil liberates not only 2-OG but in addition 2 oleic acid molecules, which may also stimulate incretin secretion. However, the previous reported effect of diet oil and the present effect of C8-diet oil giving the same full GLP-1 response as olive oil, suggests that it is mediated by 2-OG and not by fatty acids.

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Glucose spikes of eating vegetables before carbohydrates with snacks at mid-afternoon are lower than that of eating carbohydrates before vegetables without snacks

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Background and aims: Postprandial plasma glucose and glucose spikes are associated with cardiovascular diseases in patients with diabetes. An appropriate diet is needed to reduce postprandial hyperglycemia and minimize hypoglycemic events in addition to improving mean blood glucose. However, a compliance of diet in patients with diabetes is low, partly as a result of eating snacks. We evaluated whether eating order without snacks and with snacks at different time of the day could change the postprandial glucose values assessed by continuous glucose monitoring system (CGMS) in Japanese patients with type 2 diabetes mellitus (T2DM).

Materials and methods: This was a randomized, controlled, four-treatment, crossover study. Thirteen patients with T2DM (69.1 ± 6.7 yrs, HbA1c $7.5 \pm 1.2\%$: mean \pm SD) were assigned to use CGMS (Medtronic Minimed Gold) for 8 days (4-day each) either (1) eating vegetables first or (2) carbohydrates first and (3) eating vegetables first with additional snacks (biscuits 18 g, 75

kcal, carbohydrate 13.5 g, protein 1.2g, fat 1.8 g) at 12:30 and (4) 15:30. The test meals consist of rice/bread, meat/fish, 500 g of vegetables, and contain 21 g of dietary fiber: the energy ratio was 60, 16, and 24% from carbohydrates, protein, and fat, respectively. First, at 12:00 of the 1st day, each participant wore a CGMS at the clinic, and consumed the test meals at 7:00, 12:00, and 19:00 at home on the 2nd and the 3rd day, and at noon of the 4th day a CGMS was removed at the clinic. The patients consumed the first dish of vegetables for 5 min, then the main dishes for 5 min, and rice or bread for 5 min of the test meals or eating vice versa. Next, the patients ate vegetables before carbohydrates of the test meals at the same time and consumed snacks at 12:30 and 15:30 on the 2nd and the 3rd day. The daily glucose parameters were compared between four treatments.

Results: Mean blood glucose increased 0.67 mmol/L by adding snacks. Eating vegetables before carbohydrates with snacks at 15:30 resulted in lower incremental glucose peak (IGP) and incremental area under the glucose curve (IAUC) 0–3h of lunch and dinner compared with eating carbohydrates before vegetables without snacks (Figure).

Conclusion: Glucose spikes of eating vegetables before carbohydrates with snacks at mid-afternoon were significantly lower than that of eating carbohydrates before vegetables without snacks in patients with T2DM.

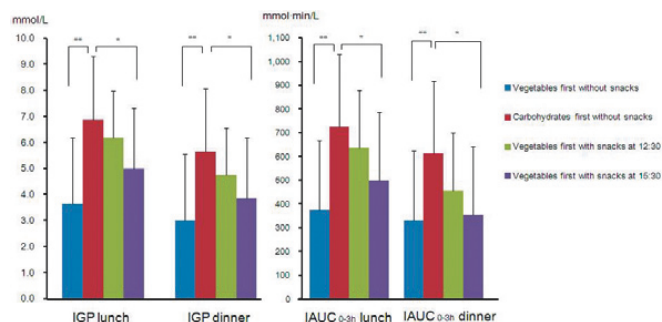


Figure IGP and glucose IAUC of lunch and dinner were reduced when the patients with T2DM consumed vegetables before carbohydrates with snacks at mid-afternoon compare to consuming carbohydrates before vegetables without snacks. Data are mean \pm SD. *** $p < 0.001$.

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Dietary carbohydrates predict maternal hyperinsulinaemia and cord blood visfatin levels in pregnancy compared to total daily caloric intake, fat and proteins

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Background and aims: Maternal diet affects insulin resistance and fetal growth-metabolism during pregnancy. Hyperinsulinemia is a marker of insulin resistance. There is still ongoing debate regarding the effect of maternal diet composition and caloric intake into maternal hyperinsulinemia and fetal metabolism. The effect of maternal dietary nutrients (carbohydrates, proteins, fat) and total daily caloric intake into maternal hyperinsulinemia, fetal metabolism and birthweight was assessed during normal pregnancy.

Materials and methods: A prospective, non interventional study, 50 pregnant non diabetic women (mean \pm SD) pre-pregnancy BMI 24.2 ± 4 kg/m² and age 29.3 ± 4.3 years were randomly assessed with validated quantitative food frequency questionnaire and a 7 day dietary diary for nutrient and total daily caloric intake (kcal) during the second trimester (24th–26th week). A 75gr oral glucose tolerance test was performed and maternal glucose and insulin levels measured at 0,5,15,30,60,90,120 min. 3 cases diagnosed with gestational diabetes were removed from the study. The delta area under the curve (Δ AUCInsulin) for maternal hyperinsulinemia was calculated by trapezoidal rule. During delivery cord blood levels for visfatin, adiponectin, leptin, glucose and insulin were measured. Birth weight was measured.

Results: Dietary analysis revealed a diet composition of 20% protein, 40% carbohydrates and 40% fat. Mean birthweight was 3132 ± 125 gr. Mean weight gain was 16 ± 3 kg. There was no association between mean maternal total caloric intake and dietary nutrients with birth weight or maternal weight. Carbohydrates and caloric intake were negatively and positively associated

with cord blood visfatin ($r = -0.91$, $p = 0.002$) ($r = 0.72$, $p = 0.037$) respectively. Backwards multiple regression analysis showed carbohydrates ($p = 0.015$, $\beta = -1.28$) being the best predictors of maternal Δ AUCInsulin of the second trimester and cord blood visfatin levels ($p = 0.009$) compared to dietary fat, proteins and mean total daily caloric intake.

Conclusion: Maternal carbohydrate intake is the best predictor of hyperinsulinemia among protein, fat and total caloric daily intake. Furthermore carbohydrate intake seems to affect the insulinomimetic secretion of adipocytokine visfatin of the fetus. Further studies need to assess the effect of maternal diet composition into fetal metabolism and growth.

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Vitamin D supplementation might increase the benefits of weight loss on insulin sensitivity in obese individuals with hypovitaminosis D

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Background and aims: Hypovitaminosis D is highly prevalent in the general population, and particularly among obese individuals. It has been suggested that, besides being associated with obesity, vitamin D insufficiency might have an independent role in the pathogenesis of insulin resistance. Healthy dietary habits and weight loss are the mainstay for the prevention of type 2 diabetes mellitus in overweight/obese individuals. Vitamin D supplementation might increase the benefits of these lifestyle interventions. Here we present the preliminary results of a currently ongoing double blind, placebo controlled randomised study. The aim of the study is to assess whether vitamin D supplementation in conjunction with a weight-loss dietary regimen has a greater impact on insulin sensitivity as compared to weight loss alone in obese subjects with hypovitaminosis D.

Materials and methods: 12 obese volunteers (8/4 F/M; mean age \pm SD: 40.4 ± 12.4 yrs; BMI: 38.3 ± 6.3 kg/m²) with hypovitaminosis D [serum 25(OH)D: 14.8 ± 7.8 ng/ml] were enrolled in this study after providing signed informed consent. Subjects were randomised (1:1) to hypocaloric diet + either oral cholecalciferol 25,000 I.U./week (VIT) or placebo (PLA) for 3 months. Anthropometric assessments, a standard 2-hr OGTT and a hyperinsulinaemic euglycaemic clamp (HEC) for the measurement of insulin sensitivity (M value) were performed at baseline and at study end.

Results: Baseline anthropometric characteristics, insulin sensitivity and 25(OH)D levels were comparable in the two groups. After 3 months of hypocaloric diet + cholecalciferol or placebo, body weight was significantly reduced in both groups (-6.3% and -9.3% for VIT and PLA, respectively; $p < 0.05$ for both; p for interaction = 0.195). 25(OH)D levels increased only in the VIT group (13.5 ± 6.2 vs. 29.1 ± 6.2 ng/ml; $p = 0.007$), and did not change in the PLA group (16.2 ± 9.5 vs. 16.1 ± 5.3 ng/ml; $p = \text{ns}$; p for interaction = 0.019). No significant differences from baseline were observed in glucose and insulin areas under the curve during the OGTT in either group. Insulin sensitivity, as assessed with the HEC, significantly improved only in the VIT group (M pre vs. post: 5.2 ± 2.4 vs. 7.3 ± 3.2 mg \cdot kg⁻¹ \cdot min⁻¹, $p = 0.046$ and 4.4 ± 2.6 vs. 4.7 ± 2.2 mg \cdot kg⁻¹ \cdot min⁻¹, $p = 0.84$ for the VIT and PLA groups, respectively). However, the increase in insulin sensitivity in the VIT group did not reach statistical significance vs. the PLA group (p for interaction = 0.161), possibly due to the relatively small number of subjects.

Conclusion: Vitamin D supplementation might potentiate the effects of weight loss on insulin sensitivity. Increasing the sample size could allow us to confirm these preliminary findings.

Clinical Trial Registration Number: NCT02020694

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Secondary metabolites from vintage vegetables improve the health status of patients with type 2 diabetes when compared to equivalent modern vegetables

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Background and aims: Vegetables are an important part of the human diet and a major source of biologically active substances called secondary metabolites. These secondary metabolites contribute to the nutritional quality of food giving it a bitter and strong taste, but more importantly are their po-

tential health promoting effects. **Aim:** To determine if a high dietary intake of bitter and strong tasting vegetables have a beneficial impact on insulin resistance related to type 2 diabetes (T2D) when compared to equivalent intake of modern mild and sweet tasting vegetables.

Materials and methods: The study was a 3-month randomized controlled parallel intervention study involving 77 participants aged 35–70 years with T2D. The participants were randomized into 3 different diets; 1) consuming daily 500 g of bitter and strong tasting (BST) vegetables 2) 500 g daily of sweet and mild tasting (SMT) vegetables and 3) normal diet (control). Both vegetable diets (group 1 and 2) consisted of root vegetables and cabbages, but the seeds for the vegetables in the BST group originate from the Nordic gene bank collection. Additionally the vegetables were ensured a bitter and strong taste through modulation of the agronomic and postharvest storage practice. **Results:** Both diets high in vegetables did significantly reduce the participants BMI ($p < 0.0001$), fasting plasma glucose ($p < 0.05$), HbA1c ($p < 0.001$), body fat composition ($p < 0.01$), fasting insulin concentration ($p < 0.05$) and HOMA-IR ($p < 0.05$). Furthermore, in the BST group significant difference was also found regarding average blood pressure from 24-h measurements ($p < 0.05$), iAUC from OGTT (insulin ($p < 0.05$) and glucose ($p < 0.01$)) and plasma lipids ($p < 0.05$).

Conclusion: The study shows that the vegetable diet with a high level of secondary metabolites hence the bitter and strong taste, have higher health promoting effects when compared to an equivalent diet with modern mild and sweet tasting vegetables. The results can be used to develop new recommendations to a healthier lifestyle and to prevent any further progression of these lifestyle diseases.

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Evaluation of the daily nutrient intake and accuracy of meal carbohydrate content estimation by type 1 diabetic patients under CSII and MDI

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Background and aims: Nutrition therapy is a key component in the treatment of people with diabetes. Carbohydrate counting is a prerequisite for making appropriate insulin therapy decisions even with the increasing use of automated bolus calculators. The goal of this study was to determine the total daily nutrient intake of carbohydrates (CHO), proteins (P) and fats (F) in conditions similar to real life. In addition, we evaluated the accuracy of meal carbohydrate estimation.

Materials and methods: Fifty type-1-diabetic patients, (26f, 24m, mean age 46 years, range: 20 to 68 years, HbA1c $7.5\% \pm 1.1\%$, duration of diabetes 23 ± 12 years, BMI 26.7 ± 4.1 kg/m², insulin therapy: CSII 35, MDI 15) participated as inpatients in the study, which lasted for 8 days. They managed their own therapy and selected their meals freely from an ample buffet of typical food choices. The entire food intake was documented during the study and full 24h intake was available for four days. The weight of the food was determined by scale, and the intake of CHO, P, and F was calculated as percentage of total calories from the nutrition labelling of the package or a nutritional software (DGE-PC vers. 2.9.1.007). Individual accuracy of CHO estimation was evaluated for a subgroup of 39 patients. They were asked to estimate the CHO content of each meal in their preferred carbohydrate estimation unit. Ratios between estimated and actual CHO content were calculated.

Results: The mean meal composition for breakfast was: 43% CHO - 16% P - 41% F as percentage of total calories (570 kcal), for lunch: 37% CHO - 20% P - 43% F (760 kcal), for dinner: 36% CHO - 21% P - 43% F (697 kcal) and for the whole day: 39% CHO ranging from 24% to 49% (45–60% recommended), 17% P ranging from 12% to 23% (10–20% recommended), 45% F ranging from 36% to 63% (25–35% recommended) (2208 kcal). When compared as percentage of the calculated CHO content, the mean estimates of the subgroup of 39 patients were in total $92.9\% \pm 13.0\%$ (median 92.4%, min. 60.3%, max. 128.8%), for breakfast $99.9\% \pm 16.9\%$, for lunch: $93.8\% \pm 16.1\%$ and for dinner: $87.2\% \pm 12.9\%$.

Conclusion: Nutrient intake was different from current nutritional recommendations of the EASD. Especially daily carbohydrate intake was lower and fat intake was higher than recommended. In previous studies we found a similar macronutrient distribution reflecting current eating patterns and personal preferences. Currently, the establishment of more individualized

recommendations is discussed. In contrast to results of other studies, the type-1-diabetic patients in this study estimated the amount of carbohydrates in self-chosen meals accurately in conditions similar to real life. Most of these patients are very motivated and participate frequently in similar studies indicating that regular training is helpful to keep nutritional education at a high level.

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The increment of postprandial energy expenditure is associated with body weight reduction by dietary intervention in type 2 diabetes

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Background and aims: Body weight change is determined by the balance between food intake and energy expenditure. Total energy expenditure is composed of basal metabolic rate (BMR), activity induced energy expenditure (AEE), and diet-induced thermogenesis (DIT). Although dietary energy restriction is an essential approach for diabetes management, the degree of body weight reduction varies between individuals. To clarify the determinants for the efficacy of dietary intervention in type 2 diabetes, we measured energy expenditure (EE) by indirect calorimeter during short-term hospitalization, and analyzed relationships between the EE and other metabolic parameters.

Materials and methods: A total of 63 subjects (21 females and 42 males) with type 2 diabetes admitted to our hospital for glycemic control were enrolled in this study. During the hospitalization (15.2 ± 2.4 days), the subjects received calorie-restricted diets (daily total energy intake was determined based on the recommendation of the Japan Diabetes Society; $[\text{height (m)}]^2 \times 22 \times 27.5$ kcal/day) with restricted exercise. Clinical measurements were carried out after an overnight fast on the 2nd day of admission. Body weight was checked again on discharge. EE was evaluated at fasting (0900 h, FEE) and postprandial state (1500 h, PPEE) using an indirect calorimeter during the hospitalization, and the increment of PPEE over FEE (Δ EE) was calculated. We analyzed correlations between the EE indices (FEE and Δ EE) and the clinical measurements. All data are expressed as means \pm SD. Mean values were compared using Student's t test. Correlations between the EE indices and other continuous variables were examined by Pearson's correlation analysis.

Results: Body weight was reduced from 67.2 ± 15.8 to 65.0 ± 15.3 kg ($p < 0.001$) during the hospitalization. PPEE was significantly higher than FEE (0.989 ± 0.208 and 1.083 ± 0.208 kcal/min for FEE and PPEE, respectively; $p < 0.001$). Δ EE was 0.094 ± 0.142 kcal/min. FEE was significantly higher in male ($P < 0.001$) and positively correlated with height ($r = 0.69$, $p < 0.001$), initial body weight ($r = 0.75$, $p < 0.001$), body mass index ($r = 0.53$, $p < 0.001$), abdominal circumference ($r = 0.52$, $p < 0.001$), fasting serum C-peptide ($r = 0.26$, $p = 0.037$) and urinary C-peptide ($r = 0.41$, $p = 0.001$), whereas inversely correlated with age ($r = -0.41$, $p < 0.001$). Δ EE showed a positive correlation with daily body weight reduction ($r = 0.37$, $p = 0.003$) and an inverse correlation with age ($r = -0.25$, $p = 0.047$). Multiple regression analysis with adjustment for covariates (age, gender, initial body weight, and HbA1c) revealed that Δ EE is a potential independent predictor for daily body weight reduction ($\beta = 0.449$, $p = 0.038$).

Conclusion: The positive correlations of FEE with anthropometric and insulin secretion parameters suggest that FEE is related to insulin resistance. On the other hand, Δ EE showed a positive correlation with body weight reduction during the short-term dietary energy restriction. Under the sedentary lifestyle like hospitalization, since FEE is nearly equivalent to BMR, Δ EE is supposed to reflect predominantly DIT. Taken together, these results indicate that Δ EE, as a surrogate marker of DIT, may determine the efficacy of dietary intervention in type 2 diabetes.

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Relationships of serum 25-OH vitamin D and parathyroid hormone levels with all-cause mortality in type 2 diabetes

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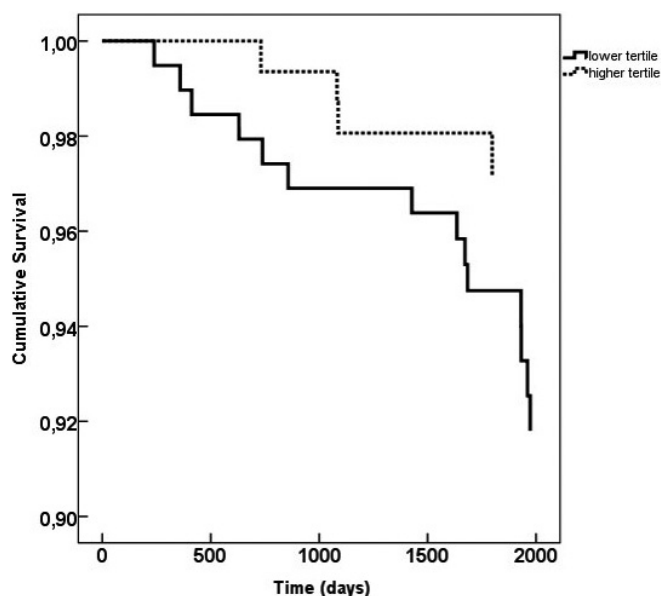
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Background and aims: New and clinically useful markers of risk are of essence in high risk states such as Type 2 diabetes.

Materials and methods: We analyzed baseline data from 741 patients (489 men and 252 women) who participated in “Cardiovascular Risk factors in Patients with Diabetes - a Prospective study in Primary care” (CARDIPP). Participants of this prospective observational study were between 55–66 years old when recruited between the years 2005–2008 and they all had Type 2 diabetes. 25-OH-vitamin D and parathyroid hormone levels (PTH) were analyzed using electrochemo-luminiscence on a Cobas® e602 unit (Roche Diagnostics Scandinavia AB, Bromma, Sweden). Carotid-femoral pulse wave velocity (PWV) was measured with applanation tonometry (SphygmoCor® system, AtCor Medical, Sydney, Australia) and intima-media thickness of the carotid arteries (IMT) was evaluated using B-mode ultrasound (ATL HDI 5000, Bothell, WA, USA). The participants were followed for all-cause mortality until December 31, 2012, using the Swedish Cause of Death Registry.

Results: There was a negative linear relationship between vitamin D levels with waist circumference ($r = -0.147$, $p < 0.0001$) in the total cohort. During the follow up period 24 men and 9 women died. When entering major risk factors in Cox regression vitamin D levels were negatively related to all-cause mortality in men, independently of age, PTH, HbA1c, waist circumference, mean 24-hour systolic ambulatory blood pressure (ABP) and apolipoprotein B levels ($p = 0.049$). This finding of increased mortality related to low levels of vitamin D in men was also statistically significant when PWV and IMT were added to the equations in men ($p = 0.028$ Hazard ratio of 0.97, CI from 0.95 to 0.997 for each nmol/l of vitamin D). The figure below shows mortality in men of the lower and higher tertiles of vitamin D levels, respectively. In women, on the other hand, levels of PTH ($p = 0.016$ without PWV and IMT) were positively related with all-cause mortality when performing the corresponding analyses ($p = 0.006$ with PWV and IMT, Hazard ratio of 1.049, CI from 1.014 to 1.085 for each ng/l of PTH) while vitamin D levels were without statistical significance in the models ($p = 0.9$).

Conclusion: Our data show that information about the levels of serum vitamin D in men and of PTH in women add prognostic information in terms of total-mortality that goes beyond traditional risk factors and that also adds information independently of the levels of systolic ABP, IMT and PWV.



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Oral glucose has no effect on appetite and satiety sensations compared to isoglycaemic i.v. glucose in healthy subjects

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Background and aims: Oral glucose ingestion is thought to elicit release of satiety-promoting gastrointestinal (GI) hormones. However, the effect of glucose stimulation of the GI tract on appetite per se has been difficult to separate from the satiety-inducing effect of elevated plasma glucose concentrations. We aimed to evaluate the GI-independent effect of glycaemic excursions following OGTT on appetite and satiety sensations in healthy volunteers.

Materials and methods: Hunger, satiety, fullness, prospective food consumption, thirst, well-being, and nausea were assessed by 100-mm visual analogue scales in 20 healthy subjects (7 females; age (mean±SEM): 54±3 years; BMI: 27.0±0.6 kg/m²; HbA_{1c} 5.4±0.1%) every 20 minutes during a 3h 50g-OGTT and an isoglycaemic i.v. glucose infusion (IIGI), respectively. Overall appetite score (OAS) was calculated as (satiety + fullness + (100 - hunger) + (100 - prospective food consumption)) / 4. Gastrointestinal-mediated glucose disposal (GIGD) was calculated as the difference in glucose amounts used during OGTT and IIGI related to the amount used during OGTT ($GIGD = 100\% \times (\text{glucose}_{OGTT} - \text{glucose}_{IIGI}) / \text{glucose}_{OGTT}$).

Results: Isoglycaemia during the oral and i.v. glucose stimuli was achieved in all subjects. As expected, glucose stimulation of the GI tract had a strong impact on glucose disposal from the blood reflected by a mean±SEM GIGD of 57±2%. No significant differences in baseline appetite and satiety sensations were observed between the two experimental days. Overall, appetite sensations increased whereas satiety sensations decreased during both glucose stimuli. There were no significant differences in hunger, satiety, fullness, prospective food consumption, thirst, well-being, nausea or OAS.

Conclusion: Our findings show that glucose stimulation of the GI tract has little or no effect on appetite and satiety sensations independently of plasma glucose levels. This suggests that the collective effect of GI factors triggered by oral glucose is of minor importance for appetite and satiety sensations in healthy subjects.

PS 059 Metformin

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Effectiveness of add-on glucose-lowering drugs for early glycaemic control in users of metformin: population-based cohort study

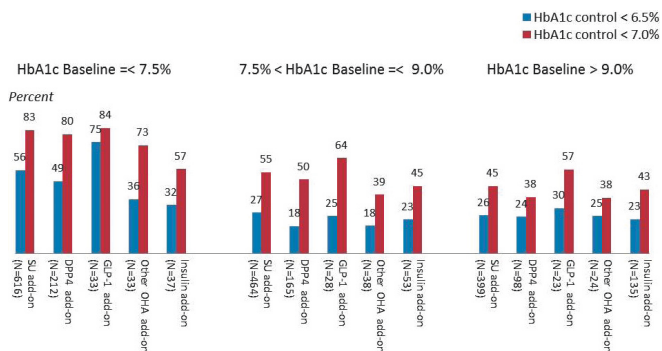
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Background and aims: We examined the association of different add-on glucose-lowering drugs with achieved quality of early glycaemic control in a large population-based cohort of incident metformin users.

Materials and methods: We included all individuals with a first-time prescription for metformin in Northern Denmark between 2000 and 2012 who had their treatment intensified with another glucose-lowering drug within the first year. We obtained individual-level data on glycated hemoglobin (HbA1c) measurements, comorbidities, and medications from population-based medical databases.

Results: We identified 25,631 T2D patients with a first metformin prescription. Of these, 3,898 (15%) received an add-on glucose-lowering drug within the first year following metformin start, of which 3,089 had a baseline HbA1c measured before their add-on drug. A number of 1,888 patients (61%) received a sulfonylurea (SU) add-on, 648 (21%) received a DPP4 inhibitor, 132 (4%) a GLP-1 receptor analogue, 125 (4%) another oral hypoglycemic agent (OHA), and 296 (10%) started insulin in addition to metformin. Median patient age at time of add-on was 60 years, 59% were males, and the median HbA1c before add-on was 7.9% (inter quartile range 7.0–9.2%). Patients with SU add-on had a median age of 61 y, a median HbA1c of 7.8% at baseline, and 23% had hospital-diagnosed comorbidity. The corresponding values were similar for patients with DPP4 inhibitor add-on (59 y, 7.7%, and 21%); for GLP-1 add-on (55 y, 8.0%, and 15%); and for other OHA add-on (59 y, 7.9%, and 18%). Insulin add-on patients tended to have worse glycaemic control and more comorbidity (56 y, 9.6%, and 29%). Within 3–6 months after add-on therapy start, 2,357 T2D patients (76%) had a control HbA1c measurement recorded. Overall, the median HbA1c dropped from 7.9% at baseline to 6.7%, corresponding to a reduction of 1.2 percentage points (pp). Absolute reductions were 1.2 pp (from 7.9 to 6.7) for sulfonylurea add-on, 1.0 pp (7.7 to 6.7) for DPP4 inhibitors, 1.5 pp (8.0 to 6.5) for GLP-1 receptor analogues, 1.1 pp (8.0 to 6.9) for other OGDs, and 2.7 pp (9.7 to 7.0) for insulin add-on. Overall, 61% of all metformin users who had add-on therapy attained an HbA1c target of <7% within 3–6 months, while 36% attained an HbA1c target of <6.5%. In the largest group with SU add-on, the proportions attaining an HbA1c target of <7% were 83%, 55%, and 45% for the 3 different baseline HbA1c levels displayed in Figure 1. In comparison, a target of <6.5% was attained by 56%, 27%, and 26%. Target attainments for other treatment groups are shown in Figure 1.

Conclusion: This study provides data on glycaemic control in real-world metformin users who are started with different add-on glucose-lowering drugs. Overall, more than one third did not attain an HbA1c target of <7% within 6 months, and close to two thirds did not attain a target of <6.5%.



Supported by: Novo Nordisk

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Cardiovascular safety of metformin and sulphonylureas in type 2 diabetes patients

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Background and aims: To investigate the impact of the exposure to the oral glucose lowering drugs (OGLD) metformin and sulphonylureas, on cardiovascular mortality in type 2 diabetes patients.

Materials and methods: All consecutive diabetes patients residing in a major urban area were screened at their first diabetes outpatient visit between 01/01/2001–12/31/2008 (n=79869). Baseline was defined at 6 months after screening (n=78714). Exclusion criteria at baseline were: insulin treatment (n=15515), not on monotherapy with metformin or sulphonylurea (n=32257), and age <40 or ≥65 years (n=14128). All 16814 patients (46.9% females) were followed-up until (i) death, based on death certificate and National Institute of Statistics data, or (ii) first insulin exposure, or (iii) end of follow-up on 12/31/2011. Data on gender, age, time-dependent daily dose and number of concomitant OGLD were available. Time-dependent competing risk regression analysis, with daily updates of treatment modalities was performed. Simultaneous use of cumulative exposure and ever exposed term of the available treatment options, a “fixed” cohort, and cumulative exposure limited to that attained one year prior to death (reverse causation) completed the evaluation.

Results: Mean baseline age was 55±6 years, with a mean follow-up of 6.0±2.9 years. During 101418 person-years exposure time, there were 968 cardiovascular deaths (328 myocardial infarction; 223 stroke) and 889 deaths from other causes (competing events), with an overall mortality rate 183 per 1000 person-years. Cumulative time exposure (one year increments) (sub) hazards (SHR) for cardiovascular death showed: male gender 1.850 (95% CI 1.618–2.115, p<0.001), age at baseline 1.083 (95% CI 1.070–1.096, p<0.001), metformin 0.979 (95% CI 0.975–0.983, p<0.001), glimepiride 0.975 (95% CI 0.968–0.983, p<0.001), gliclazide 0.978 (95% CI 0.973–0.984, p<0.001), glipizide 0.984 (95% CI 0.977–0.992, p<0.001), glibenclamide 0.985 (95% CI 0.979–0.991, p<0.001), repaglinide 0.982 (95% CI 0.970–0.995, p=0.005). There was no significant impact of pioglitazone, rosiglitazone, and concomitant number of OGLD. Cumulative dose exposure (one gram increments) results were comparable, with the exception of repaglinide 0.450 (95% CI 0.192–1.051, p=0.065) and pioglitazone 0.866 (95% CI 0.751–0.998, p=0.047). Similar results were obtained with myocardial infarction or stroke death as end-point. Future exposure to sulphonylureas in patients on metformin monotherapy at baseline (n=7546) was associated with a decrease in cardiovascular mortality. Patients originally on sulphonylurea (n=9268) and subsequently exposed to metformin did not experience significant changes in cardiovascular hazards (all SHR p values >0.05). Cumulative exposure limited to that attained one year prior to death and “fixed” cohort analysis did not affect the original results.

Conclusion: Exposure to oral glucose lowering drugs in type 2 diabetes patients originally on monotherapy with metformin or sulphonylurea is safe in terms of cardiovascular mortality. Subsequent exposure to metformin in patients treated with sulphonylurea at baseline was not associated with increased cardiovascular mortality.

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Polymorphism of OCT2 and MATE1 improve glucose-lowering effect of metformin via influencing its pharmacokinetics in Chinese type 2 diabetic patients

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Background and aims: This study aims to investigate the potential impact of organic cation transporter 2 (OCT2) nucleotide polymorphism at site 808 (G>T) and multidrug and toxin extrusion protein 1 (MATE1) on metformin pharmacokinetics and long-term antidiabetic effect.

Materials and methods: A total of 220 newly diagnosed type 2 diabetes patients taking oral metformin were recruited, genotyped and divided into three groups by SLC22A2 genotypes (G/G, G/T, T/T), as well as three groups by SLC47A1 genotypes (G/G, G/A, A/A). Six to ten patients in each group were randomly selected and undertaken metformin pharmacokinetic study. A one-year follow up randomized cohort study was performed to clarify metformin pharmacodynamics.

Results: After one year, the falling range of HbA1c level was significantly greater in subjects with heterozygous variant genotype (GT and AA) than wild type homozygote (-1.28% in GT vs -1.01% in GG, -2.32% in AA vs -1.07% in GG, both $P<0.05$) after adjustment for baseline HbA1c levels in each group. There were also differences in pharmacokinetic parameters of metformin between reference and variant forms of OCT2 and MATE1. Multivariate analysis further revealed that OCT2 exon 808 polymorphism, SLC47A1 genotype and gender were independent influencing factors for urine excretion of metformin ($P<0.05$).

Conclusion: Except for gender, the glucose-lowering efficacy of metformin can be enhanced by SLC22A2 808 G>T variants and SLC47A1 rs2289669 G>A variants through delaying its transportation and renal clearance in Chinese type 2 diabetes populations.

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Long term effects of metformin on plasma concentrations of C-peptide in insulin-treated type 2 diabetes patients: a placebo controlled trial

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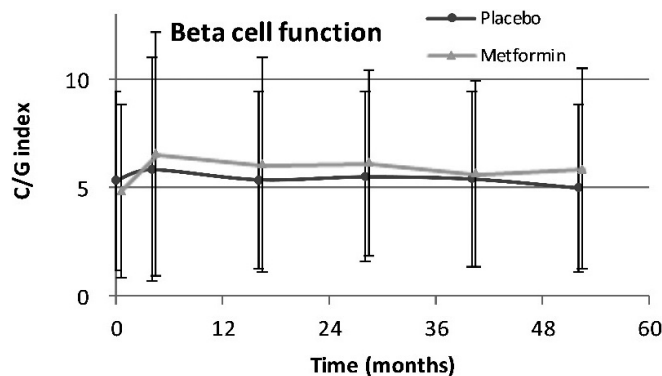
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Background and aims: Type 2 diabetes (T2D) is characterized by beta cell failure and varying degrees of insulin resistance, both being targets of treatment. While metformin is known to improve insulin sensitivity, its effects on beta cell function are assumed to be neutral. Because beta cell mass decreases over time, improvement of beta cell function may be more difficult to obtain in advanced T2D. In this study, the effects of metformin on beta cell function were evaluated in patients with advanced, insulin-treated T2D during a follow-up of 4.3 years.

Materials and methods: To evaluate the effect of metformin on endogenous insulin secretion, we analysed the plasma concentrations of C peptide (immuno-assay) at baseline, after 4 months and yearly for 4 years in a placebo-controlled, randomized trial in insulin-treated patients with T2D (N=390, mean age 61.3 years, mean diabetes duration 13.1 years, mean baseline HbA1c 7.9%, 54% men). A linear model was performed on logtransformed values because of a skewed distribution of the data, and with adjustment for baseline values.

Results: Unadjusted mean C-peptide levels decreased from 0.52 (+/- 0.38) to 0.47 (+/- 0.34) nmol/l in the placebo group, and increased from 0.47 (+/- 0.40) to 0.55 (+/- 0.47) nmol/l in the metformin group. For the final visit, regression analysis showed a significant treatment effect (difference, 5.8% (95% CI, 1.0-11.5), $P=0.034$). The summary mean in the placebo group was 0.52 (+/- 0.35) and in the treatment group 0.53 (+/- 0.40) nmol/l. No statistically significant treatment effect was found on the summary mean (difference, 2.7% (95% CI, -0.5-5.9), $P=0.101$). As a measure for steady state beta cell function, we calculated the fasting C-peptide/fasting glucose index (C-peptide/glucose x 100). This ratio decreased from 5.4 (+/- 4.1) to 5.0 (+/- 3.9) in the placebo group, and increased from 4.9 (+/- 4.0) to 5.9 (+/- 4.7) in the metformin group. Regression analysis showed a significant treatment effect both for the final visit (difference 0.9 (95% CI, 0.2-1.6), $P=0.010$) and for the summary mean (difference 0.9 (95% CI, 0.5-1.4), $P<0.001$). The greater part of this effect (71% (95% CI, 44-86)) was not mediated by changes in HbA1c.

Conclusion: Our study shows that beta cell function in advanced T2D can be improved by the addition of metformin in insulin-treated patients. This improvement is only partially explained by a reduction in HbA1c. In insulin-treated patients not treated with metformin a slight decrease in beta cell function was observed during a follow-up of 4.3 y.



Clinical Trial Registration Number: NCT00375388

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Glucose-lowering effect of metformin resides in the gut not the circulation. A 12-week placebo-controlled double-blind randomised trial

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Background and aims: Metformin delayed release (Met DR) is formulated to deliver Met directly to the lower bowel where absorption is poor, resulting in low plasma Met exposure. In previous studies, the same dose of Met DR and Met immediate release (IR) comparably lowered fasting (FPG) and postprandial plasma glucose and augmented GLP-1 and PYY release but plasma Met was reduced by approximately 50% with Met DR compared to IR. Maintained glucose-lowering efficacy with reduced plasma exposure may allow for removal of the renal impairment contraindication due to reduced Met plasma exposure and subsequent lower risk of Met-associated lactic acidosis.

Materials and methods: The randomized, blinded, 12-week study compared 3 doses of Met DR (600, 800, 1000 mg) to placebo (PBO) in subjects with T2DM (n = approximately 40/group, mean age 52 years, mean BMI 33 kg/m², screening mean FPG 144 mg/dl, and mean A1C 7.2%). Subjects were on diet and exercise (11%) or washed off previous Met (89%, average dose ~1400 mg) and/or DPP-4i for 2 weeks prior to randomization. Two open-label groups of Met extended release (XR 1000, 2000 mg) were also included as a reference.

Results: FPG decreased within the first week of treatment in all groups and was stable after 4 weeks. The mean 4-12 week change in FPG from baseline was -13.5, -12.3 and -18.0 mg/dl for 600, 800 and 1000 mg Met DR, respectively, vs. -1.2 mg/dl for PBO (all $p<0.05$, median change). The mean FPG for Met XR 1000 mg (-13.3 mg/dl, $p<0.05$ vs. PBO) was similar to 600/800 mg Met DR, despite 4-9 fold higher fasting Met plasma concentrations (515 vs. 56 and 119 ng/ml, respectively). Met XR 2000 mg reduced FPG by 26.5 mg/dl ($p<0.05$ vs PBO) with a fasting Met plasma concentration of 912 ng/ml. The similar glucose-lowering effect of 600 mg Met DR and 1000 mg Met XR represents an approximately 40% shift in the dose response for Met DR, consistent with the gut being the primary site of metformin action. At Week 12, mean A1C rose by 0.45% for PBO, reflecting withdrawal of oral agents, and remained unchanged in the DR groups and 1000 mg Met XR. The mean A1C change was -0.21% for 2000 mg Met XR. GI tolerability was similar among all Met groups.

Conclusion: Maintenance of efficacy at lower doses and markedly reduced plasma Met exposure with gut restricted Met DR confirms that Met works chiefly in the gut. Importantly, Met DR could be useful to reduce risk of lactic acidosis in patients with renal impairment.

Clinical Trial Registration Number: NCT01819272

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Effects of metformin on fuel metabolism, energy expenditure and body composition in human subjects and ratsI. Tokubuchi¹, Y. Tajiri¹, S. Iwata¹, K. Hara¹, K. Yamada¹, H. Mifune²;¹Division of Endocrinology and Metabolism, ²Department of Animal Experiment, Kurume University School of Medicine, Japan.

Background and aims: Metformin is widely used to treat obese diabetics because of its beneficial effects on body weight, energy intake, and glucose regulation. Metformin phosphorylates AMP-activated protein kinase (AMPK) and exerts its effects on hyperglycemia and insulin resistance through a blockade of gluconeogenesis in the liver and an enhancement of fat oxidation in the skeletal muscle. However, results regarding effects on energy expenditure (EE), respiratory quotient (RQ) and body composition have been rather controversial. In the present study, we investigated this unsolved issue separately during either fasting or post-prandial state in both human subjects and rats.

Materials and methods: All data were expressed as means±S.D. In human study, metformin hydrochloride (1500 mg/day) was administered either 23 healthy subjects (group C: 16 males, 26±3 years old, BMI 22.0±3.4) or 13 type 2 diabetic patients (group DM: 7 males, 44±17 years old, BMI 30.9±6.2) for 2 weeks. Meal tolerance test (592 kcal, 75 g of carbohydrate, 28.5 g of fat) was performed in the morning before and after 2 weeks administration of metformin. Blood samples were collected before and 1, 2, 3 hours after the ingestion of meal, and plasma concentrations of glucose (G), triglyceride (TG), lactate (LA) and pyruvate (PA) were measured. Simultaneously expired gas analysis was performed for the measurements of EE and RQ. In animal study, male Sprague Dawley rats (13 weeks old) were fed 10 g of standard chow twice daily (at 8AM and 7PM), and drinking water either with or without metformin hydrochloride (2.5 mg/ml) was given for 2 weeks. At 15 weeks old, EE and RQ were measured in a chamber equipped with expired gas analysis system, body compositions were measured by CT scan, then they were sacrificed in either fasting or post-prandial state and blood samples were collected.

Results: In human study, by the administration of metformin for 2 weeks significant decreases of G ($P<0.01$) and TG ($P<0.05$) were observed at all points in DM and a significant decrease of G after 2 hours ($P<0.01$) in C compared to values before administration. In DM, LA and PA after meal ingestion were significantly ($P<0.05$) increased by metformin administration. Although EE did not change, RQ decreased before meal (C: $0.8\pm0.06\rightarrow0.78\pm0.05$, $P<0.05$; DM: $0.73\pm0.03\rightarrow0.71\pm0.02$, $P<0.05$) and conversely increased 2 hours after meal ingestion (C: $0.77\pm0.05\rightarrow0.8\pm0.07$, $P<0.05$; DM: $0.71\pm0.04\rightarrow0.74\pm0.04$, $P<0.05$). In animal study, LA and PA were significantly ($P<0.05$) increased in both fasting and post-prandial state by the treatment of metformin for 2 weeks. RQ decreased during fasting state in metformin-treated rats compared to control rats (0.8 ± 0.02 vs. 0.82 ± 0.03 , $P<0.05$) in spite of comparable EEs in both groups. Total fat volume was significantly decreased by the treatment of metformin (19.5 ± 2.3 vs. $25.5\pm4.1\%$, $P<0.05$) while body weight did not differ between both groups.

Conclusion: Long-term administration of metformin brought about a shift of fuel resource, especially fat oxidation during fasting state was noted in both human and animal experiments. Concomitantly, a significant decrease of fat volume was noted in animal experiment in reflection of a dominant fat utilization. Precise molecular mechanisms for these beneficial effects of metformin are now under exploration.

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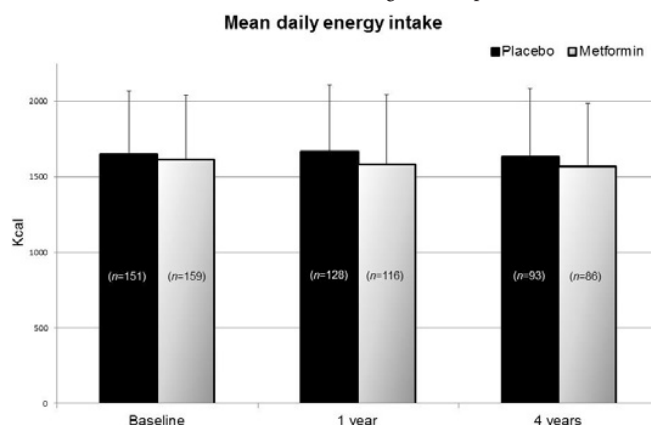
Metformin-associated prevention of weight gain in insulin-treated type 2 diabetic patients cannot be explained by decreased energy intakeM. Out^{1,2}, I. Miedema³, H.A. Jager-Wittenaar³, C.P. van der Schans³, W.P. Krijnen³, P. Leher⁴, C.D.A. Stehouwer⁵, A. Kooy^{1,2};¹Bethesda Hospital, ²Bethesda Diabetes Research Center, Hoogeveen, ³Research and Innovation Group in Health Care and Nursing, Hanze University of Applied Sciences Groningen, Netherlands, ⁴Department of Statistics, Louvain Academy, Mons, Belgium, ⁵Maastricht University Medical Centre, Netherlands.

Background and aims: Metformin is known to prevent weight gain in patients with type 2 diabetes (T2D). However, the mechanisms involved are still unknown. Less energy intake, malabsorption and metabolic effects may play a role.

Materials and methods: We analysed energy intake in the HOME trial, a placebo-controlled, randomised trial (RCT) conducted in 3 hospitals in insulin-treated T2D patients (N=390, mean age 61.3 years, mean diabetes duration 13.1 years, mean baseline HbA1c 7.9%, 54% men). Dietary intake was assessed at baseline, after 1 year and after 4 years by four trained registered dietitians. For dietary assessment, patients were asked about their intake during the preceding week according to the dietary history method. Mean energy intake per day (kcal) was calculated using food calculation software (Becel Institute Nutrition Software). All analyses were based on intention to treat. For statistical analysis, we used a general linear model (ANCOVA) and a Linear Mixed Model (LMM) to determine the robustness of our results.

Results: Dietary assessments were available from 2 hospitals, resulting in inclusion of 310 patients of the 390 patients randomised in the original study. Of the 310 participants, 179 completed (93 placebo and 86 metformin) all three dietary assessments. Body weight of the patients in the placebo group increased 4.9 kg (SD 4.9) in 4 years and increased significantly more than in the metformin group (+ 1.1 kg, SD 5.2; $p<0.001$). Mean daily energy intake after 4 years decreased from 1649 (SD 419) to 1632 kcal (SD 453) in the placebo group and from 1613 (SD 426) to 1567 kcal (SD 419) in the metformin group. ANCOVA did not detect a significant difference after 4 years (-71.0 kcal/day in metformin compared to placebo; 95% CI -199.4 to +54.5, $p=0.28$). The outcome remained non-significant after additional adjustment for gender, age, duration of diabetes and smoking, and was confirmed by a LMM (F-value 1.803, df=415, $p=0.18$). LMM failed to detect a significant effect of energy intake as explanation for the difference in weight gain between the groups (F-value 0.054, df=1, $p=0.82$).

Conclusion: In this long-term RCT in T2D patients treated with insulin, metformin treatment was not associated with a change in energy intake as compared to placebo. Therefore, the prevention of weight gain by metformin could not be explained by a lower energy intake. Further research is needed to investigate whether other mechanisms like malabsorption, changes in intestinal glucose metabolism or effects through the GLP-1 system are involved in the favourable effect of metformin on weight development.



Clinical Trial Registration Number: NCT00375388

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Are sulfonylurea and insulin therapies associated with a larger risk of cancer than metformin therapy? A retrospective database analysisB. Kowall¹, K. Kostev², W. Rathmann¹;¹German Diabetes Center, Düsseldorf, Germany,²IMS Health, Frankfurt am Main, Germany.

Background and aims: Several meta-analyses of observational studies suggested that metformin use reduces, while sulfonylurea use increases cancer risk in type 2 diabetes. However, studies of the effects of drugs are prone to bias. Recently, Suissa (2012) claimed that 23 published observational studies on the effects of metformin on cancer incidence were afflicted with time related bias like immortal time bias, bias from time lag and latency. As the few RCTs published so far have failed to confirm the results of the observational studies, the reduced cancer risk in metformin users may be put into question. Our aim is to compare cancer incidence in users of sulfonylurea, insulin and other diabetes medication, respectively, with cancer incidence in metformin users, and to avoid time related biases.

Materials and methods: In a retrospective observational study, we used the German Disease Analyzer database which includes patient data from general

practices throughout Germany. Practices anonymously report all diagnoses (ICD-10), prescriptions (ATC), hospital admissions, and laboratory test results on an ongoing basis. The study sample included 22,556 patients who received a diagnosis of type 2 diabetes during the index period used for this study (January 2000 to December 2012), and who did not have cancer before diagnosis of diabetes. The outcome measure was cancer incidence (ICD-10: all, breast, colon, and prostate cancer). The median follow-up time was 4.8 years. 1,446 (6.4%) patients developed cancer. In an intention-to-treat type analysis, patients with sulfonylurea (or insulin, or other antidiabetic medications) as their first diabetes medication were compared to patients with metformin as their first antidiabetic drug prescription. In a second type of analysis, patients with a monotherapy of sulfonylurea, insulin, or other medications, respectively, were compared to patients with a metformin monotherapy. For these analyses, time 0 was the time one year (three years in a sensitivity analysis) after the first prescription of the respective medication. Cox regression models were fitted, and hazard ratios (HR) and 95% confidence intervals (CI) were calculated to compare users of sulfonylurea, insulin, and other medications with users of metformin.

Results: Compared to patients with metformin as first diabetes medication, no increased risks of cancer of all sites were found in patients with other first diabetes medication. In models adjusted for age, sex, obesity, hypertension, hyperlipidemia, duration of diabetes, and Charlson comorbidity index, the corresponding HRs were 1.02 (95%-CI: 0.89 - 1.17) for sulfonylurea, 1.09 (95%-CI: 0.86 - 1.37) for insulin, and 0.91 (95%-CI: 0.74 - 1.12) for other diabetes medication. In the analogous analyses for the comparison of monotherapies, similar results were obtained. In additional analyses with a latency time of three years, and in analyses for some selected cancer sites (breast, colon, and prostate cancer), no decreased cancer risk was found in metformin users. **Conclusion:** In a retrospective database analysis, taking into account potential time related biases, no different cancer risk was found in metformin, sulfonylurea and insulin users. This finding is in line with few other observational studies using time-dependent analyses. To clarify the association between antidiabetic drug use and cancer risk, there is a strong need for further well-designed observational studies and RCTs.

Conclusion: Findings from the current study demonstrate that a constitutional predisposition to tumor may be enhanced by diet, and that high incidence of tumor death may be suppressed by a small dose of orally administered Metformin acting on ameliorating genes, including Ddit4 gene, in mice fed a high calorie diet. We hope this Metformin research will act as a stepping stone for making an effective drug for the anti-cancer effects especially among diabetic patients.

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The suppression mechanism of metformin on the tumour death ratio accelerated by a high calorie diet in the RasH2 mouse: a model for evaluating tumour prevention or regression

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Background and aims: The objective of the current study was to investigate whether Metformin intake changed the rate of tumor death under different diet conditions in the rasH2 transgenic mouse, a model for evaluating and designing tumor prevention or regression therapies.

Materials and methods: Male rasH2 transgenic mice at 8 weeks of age were divided into 2 groups (n=4 per group): one fed a high calorie diet of 592 kcal/100 g and drinking water *ad libitum*; and the other fed the same high calorie diet with Metformin added to the drinking water from initiation until 32 weeks. Metformin was administered at a small dose of 25 mg/kg body weight per day. Then all mice were sacrificed and examined under microarrays analysis (SurePrint G3 Mouse GE microarrays kit 8x60K, Agilent) and then quantitative PCR.

Results: Body weights of the rasH2 transgenic mice fed a high calorie diet with or without Metformin were almost the same from the age of 10 weeks until 32 weeks (76.5 ± 17.6 g vs. 76.5 ± 17.6 g). The human ras gene expression of liver, lung, kidney, spleen and visceral fat were not significantly different between the 2 groups. The lifespan of mice fed a high calorie diet with Metformin (n = 15; 75.3 ± 17.4 weeks) was longer than those fed a high calorie diet without Metformin (p < 0.05), regardless of no differences in weekly body weights. However, At autopsy, multiple occurrences of tumors in the lung, liver, spleen, digestive organs and kidney were found in all groups and malignancy was considered as the cause of death. Based on the results of microarrays analysis, we identified that Ddit4 gene, which plays an important role in responses to cellular energy levels and cellular stress, including responses to hypoxia and DNA damage, was a possible cause for this phenomenon. Our quantitative PCR experiments showed that the Ddit4 gene expression of mice with Metformin significantly decreased compared with that of mice without Metformin in kidney (0.90 ± 0.38 vs. 1.91 ± 0.49, p=0.037, n=4, respectively). Other organs showed tendency to decrease (liver: 0.35 ± 0.14 vs. 0.89 ± 0.14, p=0.09; lung: 1.43 ± 1.19 vs. 1.74 ± 2.89, p=0.64, n=4, respectively).

PS 060 SGLT2 inhibitors: safety

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Long-term renal safety with dapagliflozin treatment

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Background and aims: Dapagliflozin (DAPA) reduces hyperglycaemia by inhibiting renal glucose reabsorption. Because of this mechanism of action renal safety has been monitored closely throughout the DAPA clinical development programme.

Materials and methods: Renal safety was assessed in data pooled from 13 placebo (PBO)-controlled trials lasting up to 24 weeks (short-term [ST] dataset), 9 PBO-controlled trials lasting up to 102 weeks (ST + long-term [LT] dataset), and from all 21 phase 2b/3 trials, including an active-controlled study up to 208 weeks (all Ph2b/3 dataset) in type 2 diabetes mellitus (T2DM) patients. Frequency of adverse events (AEs), serious AEs, and abnormal laboratory tests were summarised.

Results: Among a total of 9339 patients in the all Ph2b/3 dataset, estimated glomerular filtration rate (eGFR) at baseline (BL) was ≥ 60 mL/min/1.73 m² in 8268 (88.5%) patients and ≥ 30 to < 60 mL/min/1.73 m² in 1070 (11.5%) patients. With DAPA, in the ST+LT dataset, after a small initial decrease at week 1 (mean change from BL: -4.2 vs 0.5 mL/min/1.73 m² [DAPA 10 mg vs PBO]) mean eGFR returned toward baseline by week 24 (mean change from BL: -1.4 vs -0.7 mL/min/1.73 m² [DAPA 10 mg vs PBO]) and was stable to week 102. In the ST and ST+LT datasets, AEs related to renal function were more frequent with DAPA (Table); most were small, reversible changes in serum creatinine. In both PBO- and DAPA-treated subjects, these AEs occurred > 10 times more frequently over 24 weeks, and 6–8 times more frequently over 102 weeks, in subjects with eGFR ≥ 30 to < 60 mL/min/1.73 m² at BL (moderate renal impairment) compared with those with eGFR ≥ 60 mL/min/1.73 m² at BL (Table). Serious AEs (SAEs) related to renal function were balanced across treatments and were recorded in 0.15% of patients in both DAPA and control groups in the all Ph2b/3 dataset (DAPA, 9 of 5936; control, 5 of 3403) and there were no instances of acute tubular necrosis or acute nephritis. Marked laboratory abnormalities were uncommon and similar between groups; for example, serum creatinine ≥ 2.5 mg/dL was observed in 3 (0.2%) of both DAPA- and PBO-treated patients at week 102, but in no patient with eGFR ≥ 60 mL/min/1.73 m² at BL. Shifts between categories of albuminuria (normal, micro, and macro) at week 24 in the ST dataset were similar for DAPA and PBO; overall, albuminuria did not worsen in either group. Renal safety data with DAPA were similar in a 4-year active-controlled trial (N=816).

Conclusion: Pooled data from 21 phase 2b/3 trials in T2DM patients, including 13 ST and 9 LT studies, show that DAPA 10 mg is associated with a slight excess of renal AEs, mostly transient creatinine changes, but not of renal SAEs, compared with PBO. The difference is driven primarily by the

subgroup of patients with eGFR ≥ 30 – < 60 mL/min/1.73 m² at BL. There is no indication of renal toxicity with DAPA. These data provide further evidence of a good renal safety profile for DAPA in the treatment of T2DM.

Table. Renal AEs in placebo-controlled studies up to 24 weeks and 102 weeks

Studies up to 24 weeks (13 studies, ST dataset)						
Data shown are n (%), including data after rescue	All		eGFR ≥ 60 mL/min/1.73 m ²		eGFR ≥ 30 to < 60 mL/min/1.73 m ²	
	DAPA N=2360	PBO N=2295	DAPA N=2094	PBO N=2025	DAPA N=265	PBO N=268
Most common AEs related to renal function shown individually						
AEs related to renal function	76 (3.2)	42 (1.8)	27 (1.3)	17 (0.8)	49 (18.5)	25 (9.3)
Creatinine renal clearance decreased	27 (1.1)	16 (0.7)	9 (0.4)	7 (0.3)	18 (6.8)	9 (3.4)
Renal impairment	20 (0.8)	12 (0.5)	7 (0.3)	4 (0.2)	13 (4.9)	8 (3.0)
Blood creatinine increase	15 (0.5)	9 (0.4)	4 (0.2)	3 (0.1)	11 (4.2)	6 (2.2)
GFR decrease	7 (0.3)	3 (0.1)	2 (0.1)	2 (0.1)	5 (1.9)	1 (0.4)
Studies up to 102 weeks (9 studies, ST+LT dataset)						
	DAPA N=2026	PBO N=1956	DAPA N=1774	PBO N=1705	DAPA N=251	PBO N=249
AEs related to renal function	136 (6.7)	82 (4.2)	65 (3.7)	42 (2.5)	71 (28.3)	40 (16.1)
Creatinine renal clearance decreased	46 (2.3)	28 (1.4)	21 (1.2)	15 (0.9)	25 (10.0)	13 (5.2)
Renal impairment	39 (1.9)	21 (1.1)	19 (1.1)	10 (0.6)	20 (8.0)	11 (4.4)
Blood creatinine increase	24 (1.2)	16 (0.8)	8 (0.5)	10 (0.6)	16 (6.4)	6 (2.4)
GFR decrease	11 (0.5)	8 (0.4)	5 (0.3)	4 (0.2)	6 (2.4)	4 (1.6)

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Efficacy and safety of canagliflozin (CANA) in patients with type 2 diabetes mellitus (T2DM) who progressed to stage 3A chronic kidney disease during treatment

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Background and aims: CANA, an SGLT2 inhibitor, has provided reductions in HbA1c, body weight (BW), and systolic blood pressure (SBP) across Phase 3 studies of patients with T2DM. CANA was also associated with transient reductions in estimated glomerular filtration rate (eGFR) that stabilized or attenuated over the treatment period. This analysis evaluated the impact of decreases in eGFR on the efficacy and safety of CANA in patients with T2DM.

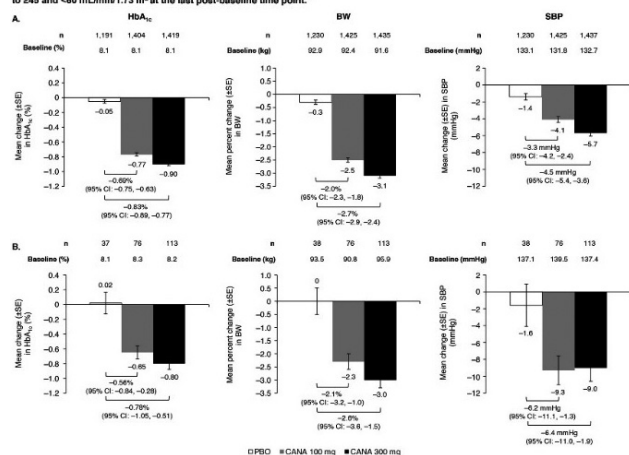
Materials and methods: Changes from baseline in HbA1c, BW, and SBP were assessed in the subset of patients with eGFR ≥ 60 mL/min/1.73 m² at baseline who had a reduction in eGFR to ≥ 45 and < 60 mL/min/1.73 m² at the last post-baseline time point based on pooled data from 6 randomised, double-blind, placebo (PBO)-controlled studies over 18 or 26 weeks (N = 262/4,158; 6.3%). Safety analyses, including the incidence of adverse events (AEs), were conducted in those who had a reduction in eGFR from ≥ 60 mL/min/1.73 m² to ≥ 45 and < 60 mL/min/1.73 m² at the last time post-baseline in a broader pooled population from 8 randomised, double-blind, PBO- and active-controlled studies over 26 or 52 weeks (N = 664/9,439; 7.0%).

Results: Among patients who had a reduction in eGFR from ≥ 60 to ≥ 45 and < 60 mL/min/1.73 m² (mean baseline eGFR 67.3 mL/min/1.73 m²), mean eGFR at the last post-baseline time point was 54.6, 54.8, and 55.9 mL/min/1.73 m² with CANA 100 and 300 mg and PBO (mean change from baseline of -12.7, -12.6, and -11.5 mL/min/1.73 m²). In these patients, CANA 100 and 300 mg provided PBO-subtracted reductions (95% confidence interval) in HbA1c (-0.56% [-0.84, -0.28] and -0.78% [-1.05, -0.51]), BW (-2.1% [-3.2, -1.0] and -2.6% [-3.6, -1.5]), and SBP (-6.2 mmHg [-11.1, -1.3] and -6.4 mmHg [-11.0, -1.9]). Changes in HbA1c and BW in patients who had eGFR reductions were similar to those in the overall efficacy population; changes in SBP with CANA were greater in those with eGFR reductions compared with the overall population (Figure). eGFR reductions were reversible once patients discontinued CANA treatment. In the broad dataset used for safety analyses, the incidence of overall AEs in patients whose eGFR decreased to ≥ 45 and < 60 mL/min/1.73 m² was 62.7%, 64.2%, and 58.6% with CANA 100 and 300 mg and non-CANA; rates of AE-related discontinuations were 6.0%,

8.1%, and 6.4% and serious AEs were 12.4%, 10.0%, and 7.9%. The incidence of volume depletion-related AEs was 4.5%, 4.6%, and 2.0% with CANA 100 and 300 mg and non-CANA; discontinuations and serious AEs related to volume depletion were infrequent across groups.

Conclusion: Among patients with T2DM with reductions from eGFR ≥ 60 mL/min/1.73 m² at baseline to eGFR ≥ 45 and <60 mL/min/1.73 m² during treatment, CANA 100 and 300 mg provided reductions in HbA_{1c}, BW, and SBP, consistent with the overall population. CANA was generally well tolerated, with a low incidence of volume depletion-related AEs across groups.

Figure. Changes in HbA_{1c}, BW, and SBP (A) in the overall population and (B) in patients with baseline eGFR ≥ 60 mL/min/1.73 m² and reduction to ≥ 45 and <60 mL/min/1.73 m² at the last post-baseline time point.*



BW, body weight; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; SE, standard error; Placebo, placebo; CANA, canagliflozin; CI, confidence interval; LS, least squares.

*PBO-subtracted differences in LS means and associated 95% CIs are shown below graphs.

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Diuresis-related safety and tolerability of dapagliflozin in type 2 diabetes mellitus over 24 weeks

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Background and aims: Dapagliflozin lowers blood glucose in Type 2 diabetes mellitus by increasing urinary glucose excretion, which is accompanied by mild osmotic diuresis.

Materials and methods: This placebo-controlled short-term pool comprised 13 double-blind core phase IIb/III studies of up to 24 weeks' duration (dapagliflozin 10 mg N=2360; placebo N=2295).

Results: The incidence of volume depletion events (events of hypotension, dehydration or hypovolaemia) was 1.1% with dapagliflozin and 0.7% with placebo. Most events were reported by investigators as being mild in intensity (0.7% and 0.4% respectively). Events in 3 dapagliflozin patients and 1 placebo patient were reported as severe by the investigator. Volume depletion events led to discontinuation in 2 dapagliflozin and 1 placebo recipient, and study drug interruption in 1 dapagliflozin and 0 placebo recipients. Events were slightly more frequent for both dapagliflozin and placebo in patients using antihypertensive therapies including diuretics, loop diuretics and angiotensin converting enzyme inhibitors/angiotensin II receptor blockers, in older patients and in patients with baseline estimated GFR ≥ 30 – <60 mL/min/1.73m² (Table). Orthostatic hypotension was measured in 13.1% of dapagliflozin recipients and 11.3% of placebo recipients, but reported as an adverse event in only 0.1% and 0.3%, respectively. Pollakiuria was reported in 2.1% and 0.7% of dapagliflozin and placebo recipients, respectively, and polyuria in 0.9% and 0.2% of patients, respectively.

Conclusion: Volume depletion events and urinary volume events were slightly more common with dapagliflozin than placebo, predominantly in at-risk subjects, but were mostly mild and did not lead to study discontinuation.

Volume Depletion Adverse Events* with Dapagliflozin		
n/N (%)	Dapagliflozin	Placebo
Overall	27/2360 (1.1)	17/2295 (0.7)
AHT medication		
Yes	26/1785 (1.5)	16/1797 (0.9)
No	1/575 (0.2)	1/498 (0.2)
Diuretic		
Yes	15/897 (1.7)	9/918 (1.0)
No	12/1463 (0.8)	8/1377 (0.6)
Loop diuretic		
Yes	6/236 (2.5)	4/267 (1.5)
No	21/2124 (1.0)	13/2028 (0.6)
ACE inhibitor/ARB		
Yes	22/1574 (1.4)	16/1577 (1.0)
No	5/786 (0.6)	1/718 (0.1)
Age		
≥75 years	3/98 (3.1)	1/81 (1.2)
≥65 years	11/665 (1.7)	6/711 (0.8)
<65 years	16/1695 (0.9)	11/1584 (0.7)
eGFR		
≥30–<60 mL/min/1.73m ²	5/265 (1.9)	4/268 (1.5)
≥60 mL/min/1.73m ²	22/2094 (1.1)	13/2025 (0.6)

ACE=angiotensin converting enzyme; AHT=antihypertensive; ARB=angiotensin II receptor blocker; eGFR=estimated GFR; *predefined Medical Dictionary for Regulatory Activities preferred terms for observed events of: hypotension, syncope, dehydration, orthostatic hypotension, blood pressure decreased, urine flow decreased, urine output decreased, and circulatory collapse

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Empagliflozin, a selective SGLT2 inhibitor, ameliorated hyperglycaemia and insulin resistance, while preserving the integrity of pancreas and kidney in CRDH rats

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Background and aims: Empagliflozin (EMPA), a selective sodium glucose cotransporter 2 inhibitor, in development for the treatment of diabetes, lowers blood glucose independently of β -cell function and insulin resistance via urinary glucose excretion (UGE). The aim of this study was to evaluate its prophylactic effects on hyperglycemia and insulin resistance in diabetic hypertensive rat model (CRDH rats).

Materials and methods: Cohen-Rosenthal diabetic hypertensive (CRDH) rats (n=36) were divided into 3 groups: sugar diet (SD) + EMPA (Empa), SD + vehicle (Veh), regular chow + vehicle (Cont). Treatment + SD were introduced concomitantly to animals 6–8 weeks old for 18 weeks. Treatment was added to drinking water and adjusted for drinking volume and body weight; daily dose of 10 mg/kg. Hyperglycemia and insulin were monitored and IP-

GTT was performed at day 80 and 120 of treatment. Immunofluorescence analysis of nephrin in the renal cortex and H&E in the pancreas was performed in the different experimental groups.

Results: Empa treatment enhanced significant ($P<0.05$) glucose excretion and associated urine volume as expected in comparison to control, reaching (>2000 vs. Veh <200 mg/dl, and 32.6 ± 3.9 vs. 15.1 ± 4.7 ml $P<0.01$), respectively. Post prandial and fasting hyperglycemia was significantly improved by Empa treatment, ($P<0.05$). Fasting insulin levels did not differ between groups however, a significant decrease ($P<0.05$) in obtained HOMA-IR was seen in the EMPA treated group vs. Veh and Cont groups. Empagliflozin reduced AUC of glucose during IPGTT at 80 and 120 days of treatment. At the end of treatment, EMPA preserved nephrin integrity in the kidney associated with a reduction of proteinuria but also prevented diabetes induced impairment of diaphragm structure. Pancreas H&E staining in empagliflozin treated group revealed an impressive decrease in fatty infiltration and atrophy in comparison to control.

Conclusion: In CRDH rat model, empagliflozin treatment was shown to reduce hyperglycemia, to improve insulin resistance and ameliorate diabetic induced kidney and pancreatic damage.

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Long-term empagliflozin treatment preserves beta cell function in ageing ZDF rats

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Background and aims: Type 2 diabetes (T2D) development is characterized by a progressive decline in β -cell function. Pharmacological interventions aimed at decreasing blood glucose levels by stimulating insulin secretion generally fail to prevent the decline of β -cell function and disease progression. With a mode of action independent of insulin secretion, SGLT2 inhibitors (SGLT2i) may interrupt the vicious cycle of β -cell exhaustion and delay or prevent the deterioration of β -cell function during the progression of T2D. The Zucker diabetic fatty (ZDF) rat is a preclinical T2D model exhibiting a rapid progression of the disease associated with early hyperinsulinemia followed by a decline in β -cell function leading to hypoinsulinemia and hyperglycaemia. We investigated whether empagliflozin, a potent and selective SGLT2i, could impact the disease progression of aging diabetic ZDF rats.

Materials and methods: 7-week-old ZDF rats were treated daily with empagliflozin (10mg/kg per os) or vehicle for 6 or 14 weeks. Baseline values were established in 7-week-old untreated ZDF rats. Body weight, 2h-fasted glycaemia, insulinemia and HbA1c were measured at baseline and then weekly during the 6 or 14 weeks of treatment (treated rats). Hyperglycemic clamps were performed at baseline and after 5 and 13 weeks of treatment.

Results: Plasma glucose and HbA1c levels increased from 7.3 ± 0.2 mM ($n=10$) and 3.0 ± 0.0 % ($n=10$), respectively, to 27.8 ± 0.9 mM ($n=9$; $p<0.001$) and 10.7 ± 0.5 % ($n=9$; $p<0.001$) over the 14 weeks study duration in vehicle-treated ZDF rats. In contrast, plasma glucose and HbA1c did not significantly increase in empagliflozin-treated rats (blood glucose: 8.0 ± 0.3 mM at 14 weeks vs. 7.0 ± 0.2 mM at 7 weeks, $n=6$, NS; HbA1c: 4.1 ± 0.2 % at 14 weeks vs. 3.0 ± 0.1 % at 7 weeks, $n=6$, NS). Insulin levels declined greatly in the vehicle-treated group from 229 ± 33 to 48 ± 4 uU/mL ($n=6$, $p<0.001$), while the decline was less pronounced in the empagliflozin group (from 237 ± 38 to 116 ± 21 uU/mL, $n=6$, $p<0.001$). During hyperglycemic clamps after 14 weeks treatment, plasma insulin levels (AUC) and maximal insulin release in response to arginine (AIRmax) were significantly higher in empagliflozin- vs. vehicle-treated groups (AUC: 174 ± 20 vs. 47 ± 4 , $n=4$ and 5 , $p<0.001$; AIRmax: 130 ± 10 vs. 22 ± 4 ng/mL, $n=4$ and 5 , $p<0.001$). Accordingly, C-peptide levels during the clamp were higher in empagliflozin-treated rats (5.6 ± 0.3 vs. 2.7 ± 0.3 nmol/L, $n=4$ and 5 , $p<0.001$).

Conclusion: Empagliflozin treatment delays the onset of hyperglycaemia in the aging ZDF rats by preserving insulin secretory capacity. This suggests that this new mode of action to decrease plasma glucose levels may prove beneficial for T2D patients by preserving β -cell function during the progression of the disease.

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LX4211, a dual inhibitor of SGLT1/SGLT2, reduces postprandial glucose in patients with type 2 diabetes mellitus and moderate to severe renal impairment

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Background and aims: The prevalence of renal impairment (RI) in type 2 diabetes mellitus (T2DM) is $\geq 20\%$. Since selective sodium-glucose co-transporter 2 (SGLT2) inhibitors target only the kidney, they have reduced efficacy in T2DM patients with RI. Because LX4211 blocks both SGLT2-mediated renal glucose reabsorption and SGLT1-mediated gastrointestinal glucose absorption, it should benefit patients with T2DM and RI by significantly reducing postprandial glucose (PPG) levels.

Materials and methods: The primary objective was to evaluate the effect of LX4211 on 4-hour PPG AUC change from baseline to Day 7 in patients with T2DM and baseline renal function (eGFR) ≥ 15 and ≤ 59 mL/min/1.73 m² (calculated by MDRD). Patients ($N=31$) were randomly assigned to receive LX4211 (400 mg, $n=16$) or placebo ($n=15$) 15 minutes before a standard breakfast on 7 consecutive days. Glucose and GLP-1 were measured 15 minutes prior to breakfast and 1, 2, 2.5, 3, and 4 hours post breakfast at baseline and on Day 7.

Results: LX4211 significantly reduced mean PPG AUC_(-15min - 4hr) by 169.3 mg•hr/dL compared to placebo in all treated patients, $p=0.003$. In patients with baseline eGFR values <45 mL/min/1.73 m², the LX4211-treated patients ($N=5$) had a 259.6 mg•hr/dL mean PPG reduction compared to the placebo patients ($N=9$), $p=0.002$. Compared to placebo, LX4211 also showed significant elevations in the mean change in incremental AUC between baseline and Day 7 for total GLP-1 of 9.7 pmol•hr/L ($p=0.017$) and for active GLP-1 of 4.7 pmol•hr/L ($p=0.042$) in all patients. There were no serious adverse events (SAEs) and no discontinuations due to AEs. There were 3 mild cases of hypoglycemia reported as treatment-emergent adverse events during the trial: 1 in the LX4211-treated patients and 2 in placebo patients.

Conclusion: These results indicate that LX4211 may enhance glycemic control in patients with moderate to severe RI.

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Dapagliflozin, a selective sodium-glucose cotransporter-2 inhibitor, does not increase risk of fractures

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Background and aims: Observational studies have shown a higher risk of fractures in patients with type 2 diabetes mellitus (T2DM), and a higher incidence of fractures versus comparator has been reported for the sodium-glucose cotransporter-2 inhibitor (SGLT2) inhibitor canagliflozin. Dapagliflozin is a highly specific SGLT2 inhibitor improving glycaemic control in T2DM by reducing renal glucose reabsorption. Here we assess frequency of fractures across the dapagliflozin clinical trial programme.

Materials and methods: Data on fractures were pooled across 21 double-blind, core phase IIb/III T2DM studies of up to 208 weeks' duration (dapagliflozin $N=5936$; placebo $N=3403$). Long-term data from a placebo-controlled subset of this pool were also analyzed, overall and in the subgroups of patients at risk: with renal impairment (estimated GFR [eGFR] using modification of diet in renal disease [MDRD] <60 mL/min/1.73m²), older patients, and women over 50 years old.

Results: Proportion of patients with fractures was numerically the same or lower in the dapagliflozin arms in all pools including patients at higher risk (Table). A dedicated study of patients with moderate renal impairment (eGFR by MDRD <60 mL/min/1.73m²) had an imbalance in fractures (8 in dapagliflozin 10 mg arm vs 0 in placebo arm). This was not confirmed in the pooled data set of patients with renal impairment.

Conclusion: Consistent with the lack of effect on bone mineral density previously reported, in these pooled clinical data analyses dapagliflozin therapy was not associated with an increase in fractures overall or in subgroups at risk (i.e. older patients, postmenopausal women and patients with renal impairment).

Table: Proportion of patients with fractures overall and in higher risk subgroups		
	Patients with fractures, N (%)	
All Phase IIb and III pool (up to 208 weeks)	DAPA total (N = 5936)	Control (N = 3403)
	79 (1.3)	53 (1.6)
Placebo-controlled pool (up to 104 weeks)	DAPA 10 mg (N = 2026)	Placebo (N = 1956)
	23 (1.1)	32 (1.6)
Subgroups at risk:		
Age ≥65 years	7/620 (1.1)	18/655 (2.7)
Age ≥75 years	1/97 (1.0)	2/77 (2.6)
Women >50 years	11/705 (1.6)	19/665 (2.9)
eGFR <60*	1/251 (0.4)	5/249 (2.0)

*mL/min/1.73m². DAPA, dapagliflozin.

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Two-year efficacy and safety of dapagliflozin for patients with type 2 diabetes mellitus and a history of cardiovascular disease
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Background and aims: Dapagliflozin (DAPA) is a selective sodium-glucose co-transporter 2 inhibitor with glucuretic and osmotic diuretic effects. The benefit/risk profile of DAPA was evaluated in a double-blind, placebo (PBO)-controlled phase 3 study of patients (pts) with type 2 diabetes mellitus (T2DM) inadequately controlled on usual care and preexisting cardiovascular disease (CVD). Primary outcome results for 24 weeks (wks) plus a 28-wk extension were previously reported; here we report results for 104 wks.

Materials and methods: Pts (HbA1c 7%–10%) were randomised 1:1 to receive 10 mg/d DAPA or PBO for 24 weeks, followed by consecutive extension periods of 28 and 52 wks. Pts were stratified by age (< or ≥ 65 years [y]) and insulin (INS) use; INS doses were reduced by 25% at randomisation. Efficacy data for 104 wks (observed cases) are descriptive (mean [95% CI]); glycaemic end points exclude pts who received rescue therapy (DAPA, n=130 [27.5%]; PBO, n=245 [50.5%]).

Results: In the efficacy population (N=962), mean age was 64 y; 47% were ≥ 65 y. Mean T2DM duration was 13 y. CVD history was mainly CHD (76.5%); 93% of pts had a history of hypertension. Most pts reported using 1 (45.4%) or 2 (34.7%) oral antihyperglycemic agents; 60.5% of patients used INS, 20% as monotherapy. Of the 964 randomised pts, 157 (33.6%) and 162 (32.5%) in the DAPA and PBO groups, respectively, were eligible to continue into the 52-week extension period. Over 104 wks DAPA maintained reductions vs PBO for HbA1c, body weight (BW), systolic blood pressure (SBP), and fasting plasma glucose (FPG) (Table). Pts in the DAPA group showed a mean decrease of -8.7 IU/d in daily INS dose compared with PBO. A larger proportion of pts achieved a combined reduction of HbA1c ≥ 0.5%, BW ≥ 3% and SBP ≥ 3 mm Hg with DAPA compared with PBO (4.2% vs 1.1%); proportions were lower in both groups compared with earlier time points. In the safety population (N=965), for DAPA vs (vs) PBO, ≥ 1 adverse event was observed in 77.0% vs 72.5% of

pts, hypoglycaemic events in 30.7% (none major) vs 29.4% (2 major) of pts, and cardiac disorders in 10.0% vs 13.5% of pts, respectively. Events of genital infection were reported in 7.7% of DAPA vs 0.4% of PBO-treated pts; events of urinary tract infection were reported in 11.8% of DAPA vs 6.8% of PBO-treated pts. For DAPA vs PBO, mean percent changes were 3.6 vs -2.0 mg/dL in total cholesterol, 4.3 vs -1.3 mg/dL in HDL-C, and 5.5 vs -1.6 mg/dL in LDL-C, respectively.

Conclusion: When added to standard of care over 104 wks in older pts with advanced T2DM and preexisting CVD, DAPA treatment resulted in sustained reductions in HbA1c, FPG, SBP, and BW. Safety and tolerability were similar to PBO, except for a small excess of genital and urinary infections.

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Outcome Measure	Placebo (N=482)	Dapagliflozin (N=480)
*n is the number of pts (full analysis set) with non-missing baseline and week 104 values		
HbA1c, %		
n*	45	78
Change from baseline, adjusted mean (95% CI)	-0.18 (-0.39 to 0.02)	-0.37 (-0.53 to -0.20)
Difference vs placebo (95% CI)	-0.18 (-0.44 to 0.07)	
Weight, kg		
n*	133	141
Change from baseline, adjusted mean (95% CI)	-0.62 (-1.25 to 0.00)	-3.35 (-3.96 to -2.75)
Difference vs placebo (95% CI)	-2.73 (-3.60 to -1.86)	
Seated systolic BP, mm Hg		
n*	102	103
Change from baseline, adjusted mean (95% CI)	-0.37 (-2.20 to 1.47)	-1.96 (-3.78 to -0.14)
Difference vs placebo (95% CI)	-1.60 (-4.18 to 0.99)	
Fasting plasma glucose, mmol/L		
n*	71	74
Change from baseline, adjusted mean (95% CI)	0.09 (-0.46 to 0.63)	-0.56 (-0.98 to -0.14)
Difference vs placebo (95% CI)	-0.65 (-1.33 to -0.03)	
Analyses exclude data after glyemic rescue and include data after antihypertensive rescue for HbA1c and FPG; include data after glyemic rescue and antihypertensive rescue for body weight endpoints, and include data after glyemic rescue and exclude data after antihypertensive rescue for seated systolic SBP endpoints.		

Clinical Trial Registration Number: NCT01042977

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Safety and tolerability of empagliflozin (EMPA) in phase III trials and their extensions in patients with type 2 diabetes (T2DM)

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Background and aims: Four 24-week randomized, double-blind, Phase III trials assessed the efficacy and safety of EMPA vs placebo (PBO) as monotherapy, add-on to metformin, add-on to metformin plus sulphonylurea, and add-on to pioglitazone ± metformin in patients with T2DM. Patients had the option to continue in 52-week double-blind extension trials. We assessed the safety and tolerability of EMPA using pooled data from these trials.

Materials and methods: In every trial, patients received EMPA 10 mg, EMPA 25 mg, or PBO until the last patient who entered the extensions had been treated for 76 weeks. Data were pooled from 2477 patients (N=825 on PBO, N=830 on EMPA 10 mg, N=822 on EMPA 25 mg) of whom 68.6% were treated in the extensions. Adverse events (AEs) were assessed descriptively in patients who took ≥1 dose of study drug. AEs included confirmed hypoglycaemic AEs (plasma glucose ≤70mg/dl and/or requiring assistance), AEs consistent with urinary tract infection (UTI), genital infection and volume depletion, identified using prospectively defined searches of investigator-reported AEs based on 77, 89 and 8 MedDRA preferred terms, respectively, and hepatic events identified from 4 Standardised MedDRA Queries.

Results: Total exposure was 1029, 1202 and 1162 patient-years in the PBO, EMPA 10 mg and EMPA 25 mg groups, respectively. The percentages of patients with any AE(s), with serious AE(s), and with AE(s) leading to treatment discontinuation were similar in the PBO, EMPA 10 mg and EMPA 25 mg groups (Table). Confirmed hypoglycaemic AEs were reported in slightly higher percentages of patients on EMPA 10 mg or EMPA 25 mg than PBO, but very few required assistance. Events consistent with UTI were reported by similar percentages of patients on PBO, EMPA 10 mg and EMPA 25 mg, and by more female than male patients. Events consistent with genital infection

were reported by a higher percentage of patients on EMPA than PBO, and by more female than male patients. AEs consistent with volume depletion were reported by 2 patients (0.2%) on PBO, 10 (1.2%) on EMPA 10 mg and 6 (0.7%) on EMPA 25 mg. Bone fractures were reported by 21 patients (2.5%) on PBO, 16 (1.9%) on EMPA 10 mg and 10 (1.2%) on EMPA 25 mg. Hepatic events were reported by 28 patients (3.4%) on PBO, 15 (1.8%) on EMPA 10 mg and 25 (3.0%) on EMPA 25 mg.

Conclusion: Based on assessment of AEs, EMPA 10 mg and EMPA 25 mg as monotherapy or add-on therapy for ≥ 76 weeks were well tolerated in patients with T2DM.

	Placebo (N=825)	Empagliflozin 10 mg (N=830)	Empagliflozin 25 mg (N=822)
Patients with AE(s), n (%)	654 (79.3)	655 (78.9)	644 (78.3)
Patients with serious AE(s), n (%)	89 (10.8)	86 (10.4)	72 (8.8)
Patients with AE(s) leading to treatment discontinuation, n (%)	48 (5.8)	33 (4.0)	44 (5.4)
Patients with confirmed hypoglycaemic AE(s), n (%)	51 (6.2)	67 (8.1)	58 (7.1)
Patients with confirmed hypoglycaemic AE(s) that required assistance, n (%)	2 (0.2)	3 (0.4)	1 (0.1)
Patients with AE(s) consistent with UTI, n (%)	133 (16.1)	127 (15.3)	114 (13.9)
Female, n/N (%)	108/401 (26.9)	107/367 (29.2)	96/358 (26.8)
Male, n/N (%)	25/424 (5.9)	20/463 (4.3)	18/464 (3.9)
Patients with AE(s) consistent with genital infection, n (%)	12 (1.5)	58 (7.0)	54 (6.6)
Female, n/N (%)	8/401 (2.0)	38/367 (10.4)	42/358 (11.7)
Male, n/N (%)	4/424 (0.9)	20/463 (4.3)	12/464 (2.6)

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Reduced risk of hypoglycaemic events with dapagliflozin vs glipizide as add-on therapy in type 2 diabetes mellitus: 4-year data from a phase 3 study

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Background and aims: Dapagliflozin (DAPA), a selective inhibitor of the sodium-glucose co-transporter 2, reduces plasma glucose by increasing renal glucose excretion. Its insulin-independent mechanism results in a low risk of hypoglycaemia in contrast to sulphonylureas. In a phase 3, randomised, double-blind trial of DAPA versus glipizide in combination with metformin (MET) in adults with type 2 diabetes mellitus inadequately controlled by MET alone (N=814), DAPA was associated with reduced hypoglycaemic events versus glipizide over 52 weeks. We now report the 4-year hypoglycaemia- and key safety-related data.

Materials and methods: The phase 3, randomised, double-blind trial of DAPA (≤ 10 mg/d) versus glipizide (≤ 20 mg/d) in combination with MET (median 2000 mg/d) was extended from 52 weeks to 4 years. DAPA and glipizide were down-titrated if medically indicated.

Results: At the start of the study, 406 patients were randomised to the DAPA arm and 408 patients to the glipizide arm. The 4-year study was completed by 161 patients in the DAPA arm and by 141 patients in the glipizide arm. Including data after rescue, the rate of hypoglycaemia was ~10-fold less with DAPA (Table). Most events were minor (capillary or plasma glucose < 3.5 mmol/L [< 63 mg/dL]). The majority of hypoglycaemic events occurred during the first year of treatment: in year 1, 3.4% versus 39.7%, year 2, 1.6% versus 23.6%, year 3, 1.5% versus 37.2%, and in year 4, 2.2% versus 28.4% in the DAPA and glipizide arms, respectively. Study medication was down-titrated in the DAPA arm only during year 1, and in only 2.7% of patients. In the glipizide arm, 15.8% of patients had a down-titration in year 1, 1.9% in year 2, and 4.8% in years 3 and 4. Key adverse events are also shown in the Table. The effect of therapy on HbA1c attenuated over time in both arms, but DAPA showed more persistent benefits versus glipizide up to year 4 (Table).

Conclusion: Fewer patients experienced hypoglycaemic events over 4 years with DAPA than with glipizide as add-on therapy to MET. DAPA treatment in combination with MET was efficacious and well tolerated over a 4-year period.

	Glipizide + MET N=408	DAPA + MET N=406
Adverse events¹		
At least 1 hypoglycaemic event, n (%)	210 (51.5)	22 (5.4)
Major hypoglycaemic event, n (%) ²	3 (7.0)	0
Discontinuation due to hypoglycaemic events, n (%)	7 (1.7)	0
At least 1 AE, n (%)	355 (87.0)	356 (87.7)
At least 1 SAE, n (%)	81 (19.9)	75 (18.5)
Deaths, n (%)	5 (1.2)	2 (0.5)
Genital infections, n (%)	12 (2.9)	58 (14.3)
Urinary tract infections, n (%)	38 (9.3)	55 (13.5)
Kidney infections, n (%)	3 (7.0)	1 (0.2)
Efficacy		
HbA1c, ³ change from baseline, % Difference (95% CI)	+0.2	-0.1 -0.30 (-0.51 to -0.09)
Weight, ³ change from baseline ⁴ , kg Difference (95% CI)	+1.12	-3.95 -5.07 (-6.21 to -3.93)
SBP, ³ change from baseline, mm Hg Difference (95% CI)	-0.02	-3.69 -3.67 (-5.92 to -1.41)

AE, adverse event; SAE, serious AE; SBP, systolic blood pressure.

¹Includes safety analysis population with data after rescue; ²Symptomatic episode needing external assistance with capillary or plasma glucose < 3.0 mmol/L (< 54 mg/dL); ³adjusted mean change; ⁴excluding data after rescue.

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PS 061 Insulin secretagogues

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Risk factors associated with treatment discontinuation and down-titration in type 2 diabetes patients treated with sulfonylureas

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Background and aims: Sulfonylurea (SU) therapy can be discontinued or down-titrated for reasons such as hypoglycemia or weight gain. A retrospective cohort study using the MarketScan database was conducted to identify risk factors associated with therapy changes (ie, discontinuation or down-titration).

Materials and methods: Patients (pts) were included when the following criteria were satisfied: first SU prescription (Rx) (index date) between 2009 and 2011, ≥ 18 years of age on the index date, and ≥ 1 year continuous enrollment pre- and post-index. Pts with type 1, gestational or secondary diabetes, insulin use before the index date, or ≥ 2 SUs on the index date were excluded. Therapy changes were determined during the 1 year post-index period. Discontinuation occurred when the gap between the end date of current SU fill and the start date of subsequent fill was ≥ 90 days apart. Down-titration occurred when an SU fill had a lower equivalent dose than that on the index date. The Kaplan-Meier method was used to estimate 3- and 6-month therapy change rates. Cox regression was used to identify risk factors associated with therapy changes in hazard ratios (HRs).

Results: 104,082 pts were included, of which 55,233 (53.1%) experienced therapy changes in the 1-year post-index period. 3- and 6-month therapy change rates were 23.2% and 38.9%, respectively. Major risk factors associated with therapy changes were post-index hypoglycemic events (discontinuation HR= 1.78 [1.68, 1.90], $p<.01$; down-titration HR=2.79 [2.40, 3.23], $p<.01$) and concomitant use of insulin (discontinuation HR= 1.48 [1.40, 1.57], $p<.01$; down-titration HR=1.82 [1.56, 2.11], $p<.01$). Other risk factors include younger age, use of 2nd generation SUs, prior cardiovascular conditions and liver disease.

Conclusion: More than half of type 2 diabetes pts who newly initiated SU therapy experienced discontinuation or down-titration within 1 year following treatment initiation. Insulin use and hypoglycemic events elevated the risk of therapy changes.

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Severe hypoglycaemia during treatment with sulphonylurea in patients with type 2 diabetes in the capital region of Copenhagen, Denmark

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Background and aims: Sulphonylureas (SU) are still recommended as a well established second line treatment in current guidelines for type 2-diabetes (T2DM). 16.865 patients in the Capital Region of Copenhagen were given SU as part of their treatment of T2DM in 2010-2011. Hypoglycaemia is a well-known side-effect, however to what extent SUs are associated with hospitalizations due to severe hypoglycaemic episodes, defined as episodes with a need for external assistance, less is known. The aim of the present study was to investigate the prevalence and characterize patients with type 2-diabetes treated with SU and hospitalized due to hypoglycaemia.

Materials and methods: All patients with ICD-10 diagnosis codes of hypoglycaemia and/or T2DM, in the hospital admittance register for a period of two years (2010-2011), were included. All hospitals in the Capital Region of Copenhagen were included. From the hospital records, patients who met the inclusion criteria were included in the analysis. Inclusion criteria were T2DM, hospitalization due to hypoglycemia and treatment with SU as monotherapy or in combination with other glucose-lowering drugs, excluding insulin treatment.

Results: From the hospital admittance register we found 3.156 patients admitted to hospital with T2DM and/or hypoglycaemia. Of these, 163 patients

fulfilled the inclusion criteria. The mean age was 76 (53-97) years and 54% were males. Nearly half of the patients were treated with SU monotherapy, 45% were treated with SU and metformin and 6% with triple therapy. Among the patients, 74% were on glimepiride, 15% glibenclamide, 6% glipizide and 2% gliclazide treatment. Sixty percent of the patients had diabetic complications, including 19% with diabetic nephropathy. The major reason for the severe hypoglycaemic episodes was unchanged dose of SU despite of a significant decline in food intake (57%). In 10% of the patients (over)use of other medications, such as benzodiazepine and opioid drugs were recorded. In 6% of the patients intoxication by alcohol was noted as reason. A concomitant infection existed in 5%. In 22% of the patients more than one reason was listed, most common were unchanged dose of SU despite of a significant decline in food intake and concomitant infection. In 22%, the reason for hypoglycaemia was unknown.

Conclusion: In conclusion, the incidence of hospital admission-requiring severe hypoglycaemia in patients treated with SU seems relatively low (below 1% of SU-treated patients). It was mainly older patients with diminished food intake, (over) use of alcohol or other medications, and with concomitant diabetic complications.

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HNF1A and HNF4A mutations are associated with prolonged half-life of glibenclamide but not glipizide in human MODY subjects

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Background and aims: Patients with Maturity Onset Diabetes of the Young (MODY), which is caused by mutations in the transcriptional factors HNF-1 α or HNF-4 α , display a hypersensitivity to the hypoglycaemic effects of sulfonylurea derivatives. The transcriptional network, including HNF-1 α and HNF-4 α , synergistically up-regulates the transcriptional activity of human xenobiotic-inducible genes, such as CYP2C9, which ensures the biotransformation of sulfonylurea derivatives in the liver. We hypothesized that MODY-associated HNF1A/HNF4A mutations induce the reduction of sulfonylurea derivatives clearance, thereby prolonging their action and promoting hypersensitivity in affected patients.

Materials and methods: Single doses of 3mg glipizide and 5mg glibenclamide were administered sequentially to seven MODY subjects (six HNF1A-MODY and one HNF4A-MODY) and six age- and BMI-matched individuals with the wild-type sequence of HNF1A/HNF4A exons. Pharmacokinetics (plasma concentration levels, C_{max}, t_{max}, t_{1/2}, AUC) and pharmacodynamics parameters (glycaemia, C-peptide and insulin plasma levels) were followed for 24 hours after the drug administration. CYP2C9 genotype was tested in each subject.

Results: Following glipizide treatment, there were no significant decreases in any of the pharmacokinetic parameters as measured when comparing MODY and control subjects. In contrast, the responses to glibenclamide treatment displayed a prolonged t_{1/2} from 5.0 \pm 1.4h in control subjects to 9.5 \pm 6.7h in HNF1A/HNF4A MODY subjects ($p = 0.04$). When focusing on pharmacodynamics, we observed a differential response in control subjects to the doses of glipizide and glibenclamide applied. However, this differential response was not achieved in MODY subjects, suggesting a partially different mechanism of action of glipizide and glibenclamide.

Conclusion: It was only prolonged half-life of glibenclamide, but not glipizide, which we observed in HNF1A/HNF4A MODY subjects compared to control subjects. Importantly, the response of individual MODY subject to glipizide or glibenclamide treatment was highly correlated with each other (but not in control subjects), thereby providing a novel platform for the application of personalized medicine. Further research on the mutation-specific responses of HNF1A or HNF4A MODY subjects is needed.

Clinical Trial Registration Number: UNCE 204015 and PRVOUK P31/2012, NT13663-3/2012

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Impact of hypoglycaemic events and HbA1c level on SU discontinuation and down-titrationY. Qiu¹, P. Lares¹, C.-P. Fan², Z. Li², J. Tang², K. Iglay¹;¹Merck & Co., Inc., Whitehouse Station, ²Asclepius JT, New York, USA.

Background and aims: A retrospective cohort study using GE Centricity electronic medical records (EMR) was conducted to assess the association of hypoglycemic events and HbA1c level with discontinuation and down-titration of sulfonylureas (SUs) among patients with type 2 diabetes mellitus (DM).

Materials and methods: Patients with ≥ 12 months EMR activity before and after initial SU prescription (index date) in 2010–2012 were included. Patients were excluded if they had type 1, gestational or secondary DM, received insulin before the index date, used ≥ 2 SUs on the index date, or had incomplete prescription data in 1 year post-index. Discontinuation occurred when consecutive SU prescriptions (Rx) were ≥ 90 days apart. Down-titration occurred when a subsequent SU Rx had a lower equivalent dose than the index dose. Post-index hypoglycemic events (identified using ICD-9 codes) occurred after the index date and before therapy changes or the end of the 1 year post-index. Multivariate Cox regression was used to assess the association of post-treatment hypoglycemic events and HbA1c with discontinuation/down-titration.

Results: 28,371 patients were included in the study, of which 13,459 (47.4%) were discontinuers, 717 (2.5%) down-titrators, and 14,195 (50.0%) continuers. 0.6% of the continuers had hypoglycemic events in the 1 year post-index period, compared to 3.1% of down-titrators and 0.8% of discontinuers ($p < 0.01$). Patients with hypoglycemic events were at higher risk for discontinuation (HR = 1.82, [1.47, 2.23]; $p < 0.01$) and down-titration (HR = 4.25, [1.92, 8.03]; $p < 0.01$). Patients with higher post-index HbA1c levels or use of 2nd generation SUs (vs. 3rd generation) were at higher risk of discontinuation (HR = 1.05 [1.04, 1.06], and HR = 1.19 [1.14, 1.24] respectively, both $p < 0.01$). **Conclusion:** Hypoglycemic events were a significant risk factor for SU discontinuation or down-titration. Higher HbA1c levels were significantly associated with SU discontinuation.

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Efficacy and safety of trelagliptin, a novel once-weekly oral DPP-4 inhibitor: a phase 3, double-blind, non-inferiority study in Japanese type 2 diabetes mellitus patientsN. Inagaki¹, H. Onouchi², H. Sano², S. Kuroda², K. Kaku³;¹Department of Diabetes, Endocrinology and Nutrition, Kyoto University Graduate School of Medicine, ²Pharmaceutical Development Division, Takeda Pharmaceutical Company Limited, Osaka,³Kawasaki Medical School, Okayama, Japan.

Background and aims: There are some options of oral hypoglycaemic agents for type 2 diabetes mellitus (T2DM). In patients with T2DM, improving adherence to medication is important in order to maintain favourable glycaemic control during long-term treatment and, thus prevent the onset or aggravation of complications. Trelagliptin is a novel dipeptidyl peptidase-4 (DPP-4) inhibitor, which is currently under development as a once-weekly oral hypoglycaemic agent. A phase 1 single-dose study (3.125 mg to 800 mg) and a multiple-dose study (100 mg or 200 mg) in healthy adult subjects were completed, and a dose-dependent increase in pharmacokinetic parameter values was observed. Sustained Inhibition of DPP-4 activity up to 168 hours after administration was also observed. Furthermore, a phase 2 dose-ranging study (12.5 mg to 200 mg; once-weekly for 12 weeks) in T2DM patients showed a statistically significant HbA1c reduction at all doses compared with placebo. Based on these results, a phase 3 study in T2DM patients was conducted to evaluate the efficacy and safety of once-weekly trelagliptin treatment compared with a daily DPP-4 inhibitor (alogliptin).

Materials and methods: This was a phase 3, multicentre, randomised, double-blind, parallel-group, non-inferiority study to evaluate the efficacy and safety of trelagliptin 100 mg once-weekly for 24 weeks with alogliptin 25 mg daily as a comparator in Japanese T2DM patients with inadequate glycaemic control despite diet and/or exercise therapy. A placebo group was also set as a reference group. Patients were randomly assigned (allocation ratio 2:2:1) to receive either trelagliptin, alogliptin or placebo. The primary endpoint was the change from baseline in HbA1c at the end of the treatment period.

Results: A total of 243 patients were enrolled to receive trelagliptin (n=101), alogliptin (n=92), or placebo (n=50). Baseline characteristics were as fol-

lows: mean age (SD) of 58.9 (10.39) years, mean BMI of 24.96 (4.161) kg/m² and mean HbA1c of 7.78 (0.837) %. There was no major difference among the treatment groups. At the end of the treatment period, the least square mean difference (trelagliptin - alogliptin) of change from baseline in HbA1c was 0.11% (95% CI: -0.054 to 0.281). Non-inferiority of trelagliptin group to alogliptin Group was attained. Additionally, HbA1c was decreased significantly in the trelagliptin and alogliptin groups compared to the placebo group at the end of the treatment period ($p < 0.0001$). Inhibition rate of DPP-4 activity was also measured, and trelagliptin group showed sustained inhibition of DPP-4 activity throughout the treatment period. The frequency of adverse events in the trelagliptin group was similar to those in alogliptin and placebo groups. No hypoglycaemia was reported in trelagliptin group. Once-weekly treatment with trelagliptin was well tolerated and no major concern was seen.

Conclusion: In this study, once-weekly trelagliptin treatment was as effective as a daily DPP-4 inhibitor, alogliptin, and was well-tolerated over 24 weeks. It may provide a new treatment option for T2DM patients.

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Cost-effectiveness of metformin plus vildagliptin versus metformin plus sulphonylurea for the treatment of type 2 diabetes patients in PortugalD. Viriato¹, F. Calado², J.-B. Gruenberger², S.H. Ong², D. Carvalho³,J. Silva-Nunes⁴, S. Johal⁵, R. Viana¹;¹Novartis Farma SA, Porto Salvo, Portugal, ²Novartis Pharma AG, Basel,Switzerland, ³Faculty of Medicine, São João Hospital, Porto, ⁴Curry CabralHospital, Lisbon, Portugal, ⁵HERON Commercialization, London, UK.

Background and aims: The objective of the study is to evaluate the cost-effectiveness of vildagliptin plus metformin compared with generic sulphonylurea plus metformin in patients with type 2 diabetes mellitus, not controlled with metformin, from a Portuguese healthcare system perspective.

Materials and methods: A cost-effectiveness model was constructed using risk equations from the United Kingdom Prospective Diabetes Study Outcomes Model with a 10,000-patient cohort and a lifetime horizon. The model predicted microvascular and macrovascular complications and mortality in yearly cycles. Patients, who entered the model as metformin monotherapy failures, were modelled as switching to alternative treatments (metformin plus basal-bolus insulin and subsequently metformin plus intensive insulin) using a threshold of glycated haemoglobin A1c $> 7.5\%$ for inadequate glycaemic control. Baseline patient characteristics and clinical variables were derived from a Portuguese epidemiological study. Costs estimates were based on direct medical costs only. One-way and probabilistic sensitivity analyses were conducted to test the robustness of the model assumptions.

Results: Over a lifetime horizon, there were fewer non-fatal diabetes-related adverse events (AEs) in patients treated with metformin plus vildagliptin compared with patients treated with metformin plus sulphonylurea (6752 versus 6815). Addition of vildagliptin compared with sulphonylurea led to increased drug acquisition costs counterbalanced with reduced costs of AEs, managing morbidities, and monitoring patients. Treatment with metformin plus vildagliptin yielded a mean per-patient gain of 0.1279 quality-adjusted life years (QALYs) and a mean per-patient increase in total cost of €1161, giving an incremental cost-effectiveness ratio (ICER) of €9072 per QALY. Univariate analyses showed that ICER values were robust and ranged from €4195 to €16,052 per QALY when different parameters were varied. The Probabilistic Sensitivity Analysis suggested that, for a willingness-to-pay threshold of €30,000 per QALY, treatment with metformin plus vildagliptin had a 79% probability of being cost-effective compared with metformin plus sulphonylurea.

Conclusion: Treatment with metformin plus vildagliptin compared with metformin plus sulphonylurea is expected to result in a lower incidence of diabetes-related AEs and to be a cost-effective treatment strategy.

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Predictors of glycaemic response to add-on therapy with a DPP-IV inhibitor: a retrospective cohort study using the primary care THIN database

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Background and aims: Apart from baseline HbA1c, little is known about clinical or biochemical factors that affect the magnitude of the glycaemic response to a DPP-IV inhibitor when used in routine practice as part of combination therapy in patients with type 2 diabetes. Similarly, there is limited information about the effectiveness of a DPP-IV inhibitor in different drug combinations. These subjects were investigated using a large UK Primary Care database.

Materials and methods: A cohort of 25,183 patients with type 2 diabetes, newly treated with a DPP-IV inhibitor between June 2007 and May 2013, was sourced from UK General Practices via the Health Initiative Network (THIN) database. The index date was defined as the date of initiation of DPP-IV inhibitor therapy. Baseline clinical parameters of patients with suboptimal glucose control (HbA1c > 6.5% or 48 mmol/mol) 6 months or more after starting a DPP-IV inhibitor in combination with other glucose-lowering therapy (n=17,697) were compared against 12-months follow-up data using multivariate logistic regression. Response to DPP-IV inhibitor therapy was defined as HbA1c < 6.5% and/or > 1% (13 mmol/mol) reduction at 12 months. A comparative analysis of on-treatment effects of various combination regimens were examined using a propensity score matching technique. Associations were examined using t test and Chi squared test.

Results: Among patients whose glucose-lowering therapy was intensified by co-administration of a DPP-IV inhibitor, independent predictors of response were baseline HbA1c [1.57, 95%CI (1.53–1.61)], and use of metformin [1.31, 95%CI (1.20–1.44)] and aspirin [1.14, 95%CI (1.06–1.23)]. The independent predictors of non-response included obesity [0.82, 95%CI (0.72–0.93)], previous hypoglycaemia events [0.84, 95%CI (0.77–0.93)], total cholesterol level [0.94, 95%CI (0.90–0.97)], concurrent use of lipid lowering drugs [0.88, 95%CI (0.81–0.97)], and combination therapy involving metformin, sulphonylurea and thiazolidinediones (TZDs) [0.80, 95%CI (0.70–0.91)]. Overall, intensification of therapy with the addition of a DPP-IV inhibitor resulted in significant reductions in HbA1c (0.5%, 6.5 mmol/mol), body weight (1 Kg) and total cholesterol 0.1 mmol/L (p<0.001). Using propensity score analysis, the addition of a DPP-IV inhibitor to metformin monotherapy yielded a 5% greater glycaemic effect [1.05, 95%CI (1.02–1.07)] compared with other regimens, while adding a DPP-IV inhibitor to Metformin+Sulphonylurea+TZD had a negative effect on glucose control [0.95, 95%CI (0.93–0.98)].

Conclusion: This large Primary Care database shows that in routine clinical practice the glycaemic response to a DPP-IV inhibitor is greatest in patients concurrently receiving metformin and aspirin and those with higher baseline HbA1c. Lower responses were observed in those on triple therapy (Met+SU+TZD) and with previous hypoglycaemic events. Serum lipids and statin therapy may also influence the response to a DPP-IV inhibitor.

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Efficacy and safety of initial therapy with linagliptin and pioglitazone fixed-dose combinations versus monotherapy with pioglitazone or linagliptin

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Background and aims: Targeting the core defects in type 2 diabetes (T2D) by combining oral antidiabetes drugs (OAD) with different mechanisms of action may produce additive improvements in glycaemic control. This study evaluated the efficacy and safety of fixed-dose combinations (FDC) of the DPP-4 inhibitor linagliptin (LINA) plus the thiazolidinedione pioglitazone (PIO) vs LINA or PIO monotherapy.

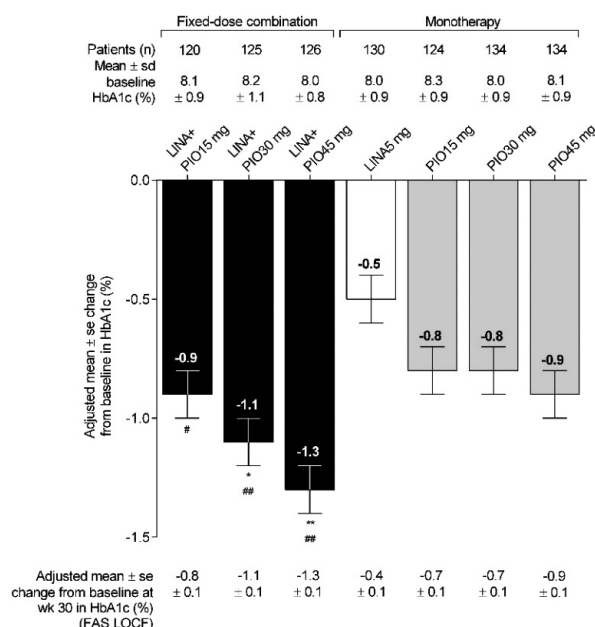
Materials and methods: This multicentre, double-blind, parallel group, phase 3 study randomised 936 T2D patients with insufficient glycaemic control to 1 of 7 treatment arms: LINA+PIO FDC (LINA5 mg plus PIO15, 30, or 45 mg) or monotherapy with LINA5 mg or PIO15, 30, or 45 mg. An initial 30-wk treatment (Part A) was followed by a 54-wk extension (Part B; 557

patients) in which LINA+PIO FDC (LINA5 mg plus PIO30 or 45 mg) were further assessed vs LINA5 mg or PIO30 or 45 mg monotherapy. The primary endpoint was HbA1c change from baseline after 30 wks (FAS LOCF).

Results: At baseline, 54.8% were male, 85.1% were white, mean ± sd age was 57 ± 11 yrs, and weight was 93.1 ± 19.5 kg. In the full analysis set (n=893), 68.1% had T2D for ≤ 5 years, 33.9% received ≥ 1 prior OAD, and mean ± sd HbA1c was 8.1 ± 0.9%. The FDC arms had larger decreases from baseline after 30 wks in HbA1c than the respective monotherapies (Part A; Figure). At 84 wks (Part B), mean ± sd change from baseline in HbA1c ranged from -0.61 ± 0.61% (LINA; n=12) to -1.30 ± 0.78% (LINA+PIO45 FDC; n=15). In patients with baseline HbA1c ≥ 7.0%, each FDC arm was more likely than LINA monotherapy to achieve HbA1c < 7.0% at wk 30 (p<0.001); in the comparison vs PIO monotherapy, only LINA+PIO45 FDC was more likely than PIO45 to achieve HbA1c < 7.0% (p=0.0254). Adjusted mean ± se changes from baseline after 30 wks in FPG ranged from -0.08 ± 0.19 mmol/l (LINA) to -1.95 ± 0.19 mmol/l (LINA+PIO45 FDC). Adjusted mean ± se body weight changes were lower with LINA monotherapy (-0.62 ± 0.76 kg) and FDC (range: +0.63 ± 0.70 kg to +1.50 ± 0.74 kg) than with PIO monotherapy (range: +1.53 ± 0.78 kg to +3.24 ± 0.73 kg). In the safety analysis (up to 84 wks from Parts A and B combined), frequencies of overall AEs and serious AEs ranged from 65.7% (PIO30) to 74.6% (PIO45) and 3.6% (PIO45) to 11.3% (LINA+PIO30 FDC), respectively. Drug-related AEs ranged from 11.1% (LINA+PIO15/30 FDC) to 16.5% (LINA+PIO45 FDC). Predefined AEs of oedema ranged from 4.4% (LINA) to 12.8% (LINA+PIO45 FDC). One on-treatment fatality was reported (PIO45). Frequency of investigator-reported hypoglycaemia was ≤ 1.5% per arm. One patient had severe hypoglycaemia (LINA+PIO15/30 FDC).

Conclusion: FDC therapy with LINA+PIO was well tolerated with no new safety concerns compared with the known safety profiles of the individual monotherapies and had possible additive effects on glycaemic control; in addition, LINA attenuated the weight gain effects of PIO.

Figure. Adjusted mean change from baseline at week 30 in HbA1c (Part A; FAS LOCF-ROC)



*p=0.0022, **p=0.0007 vs corresponding PIO monotherapy

†p=0.0017, ‡p<0.0001 vs LINA 5 mg monotherapy

Model includes fixed effects for treatment (Part A), continuous baseline HbA1c, prior OADs, and country

FAS, full analysis set; LINA, linagliptin; LOCF, last observation carried forward; OAD, oral antidiabetes drug; PIO, pioglitazone; NCT, rescue observed cases

Clinical Trial Registration Number: NCT01183013

Supported by: Boehringer Ingelheim

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Vildagliptin as add-on to insulin improves glycaemic control without increased risk of hypoglycaemia in Asian (predominantly Chinese) patients with type 2 diabetes mellitus

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Background and aims: There are limited data from randomized controlled trials evaluating the efficacy and safety of oral antidiabetic drugs in combination with insulin in Asian patients with T2DM, who may be at higher risk of hypoglycaemia due to lower weight and increased insulin sensitivity. Here we present the efficacy and safety of vildagliptin in combination with insulin with or without metformin from a dedicated Asian study in a predominantly Chinese population with T2DM.

Materials and methods: In this 24-week, multicentre, double-blind, placebo-controlled trial, patients with T2DM inadequately controlled (HbA1c 7.5–11.0%) on stable therapy with long-acting, intermediate-acting or premixed insulin, with or without concomitant metformin were randomized to receive vildagliptin 50 mg bid (n=146) or placebo (n=147). The primary efficacy endpoint was change in HbA1c from baseline to week 24. Key secondary endpoints included change in FPG and the proportion of patients achieving HbA1c <7.0%. Safety and tolerability were also assessed.

Results: Of the 293 randomized patients, 276 completed the study. The demographic and baseline characteristics of patients were comparable between the groups. At baseline the mean age of patients was 58.1 years, mean T2DM duration was 11.3 years, mean FPG was 9.5 mmol/L, mean BMI was 26.1 kg/m² and mean HbA1c was 8.7%. At baseline, 71% of patients were on stable metformin therapy. The mean doses of insulin and metformin were 32.5 U/day and 1587.7 mg/day, respectively. After 24 weeks vildagliptin showed a clinically relevant and statistically significant reduction in HbA1c vs. placebo (-1.1% vs. -0.4%, respectively; $p < 0.001$). The decrease in FPG from baseline was numerically higher in the vildagliptin group than in the placebo group (-0.72 mmol/L vs. -0.28 mmol/L; $p = 0.250$). The proportion of patients achieving HbA1c <7.0% was significantly higher in the vildagliptin group than in the placebo group (23.6% vs. 11.1%, $p < 0.005$). The overall incidences of adverse events were 43.8% vs. 46.3% and serious adverse events were 3.4% vs. 6.8% in the vildagliptin and placebo groups, respectively. Hypoglycaemia was less frequent with vildagliptin (n=4, 2.7%) than with placebo (n=8, 5.4%); one patient in the placebo group reported a severe hypoglycaemic event. No deaths were reported during the study.

Conclusion: In Asian (predominantly Chinese) patients with T2DM inadequately controlled on insulin (with or without metformin), the addition of vildagliptin 50 mg bid significantly improved glycaemic control without an increased risk of hypoglycaemia. Vildagliptin is an attractive treatment for these patients.

Clinical Trial Registration Number: NCT01582230

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PS 062 SGLT2 inhibitors: non-glycaemic endpoints

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The cost-effectiveness of canagliflozin (CANA) versus sitagliptin (SITA) as third-line therapy in the treatment of type 2 diabetes mellitus (T2DM) in the UK

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Background and aims: CANA is a novel treatment for T2DM that inhibits the sodium glucose co-transporter 2, a mechanism that is complementary to other anti-hyperglycemic drug classes, including insulin. In a previously reported analysis of a randomized, double-blind, active-controlled, Phase 3 study in 756 subjects inadequately controlled on dual therapy with metformin (MET) and sulfonylureas (SU), CANA 300mg demonstrated statistical superiority compared to SITA 100mg in lowering HbA1c at 52 weeks (-1.03% [-11.3 mmol/mol] and -0.66% [-7.2 mmol/mol], respectively; least squares mean difference -0.37% [95% CI, -0.50 to -0.25] or -4.0 mmol/mol [-5.5 to -2.7]). CANA 300mg also significantly reduced systolic blood pressure (SBP) and body weight (BW) versus SITA 100mg (SBP: -5.1 mmHg; 0.9 mmHg), (BW: -2.5%; 0.3%). The objective of this study was to evaluate the long-term cost-effectiveness of using CANA 300mg versus SITA 100mg as an add-on therapy in patients inadequately controlled on a background of MET plus SU in the UK.

Materials and methods: Outcomes and costs associated with CANA 300mg versus SITA 100mg in triple therapy with background MET plus SU were simulated over 40 years using ECHO (Economic and Health Outcomes)-T2DM, a validated micro-simulation model. Treatment effects (HbA1c, BW, SBP, LDL and HDL cholesterol) and incidence of adverse events (AEs) were sourced from the trial. Simulated treatment was intensified when HbA1c exceeded 7.5% by adding basal insulin, and subsequently prandial insulin. Costs and quality of life impacts associated with the development of micro- and macro-vascular events and AEs were adapted to the UK setting. In the base case, initial patient characteristics were sourced from the trial. In a second simulation, initial patient characteristics were based on a sample of patients with T2DM in the THIN (The Health Improvement Network) database, a large anonymised primary care electronic medical record data resource representative of UK population.

Results: In the base case simulation, use of CANA 300mg versus SITA 100mg as an add-on in patients inadequately controlled on MET plus SU increased Quality-Adjusted Life-Years (QALYs) (0.04). Total lifetime treatment costs for CANA 300mg were marginally higher (£671), yielding an incremental cost-effectiveness ratio (ICER) of £17,813. The scenario that used baseline patient characteristics from the UK THIN database also yielded an increase in QALYs (0.02), with a corresponding ICER of £17,968.

Conclusion: The magnitudes of the ICERs estimated in these simulations are below the current threshold used in the UK to assess whether an intervention is cost-effective. As such, treating patients inadequately controlled on MET plus SU with CANA 300mg is likely to be a more efficient use of limited health care resources compared to using SITA 100mg.

Clinical Trial Registration Number: NCT01137812

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Effect of empagliflozin compared with glimepiride as add-on to metformin for 2 years on the amount and distribution of body fat in patients with type 2 diabetes

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Background and aims: In a randomized, double-blind, Phase III trial in patients with type 2 diabetes (T2DM), the SGLT2 inhibitor empagliflozin (EMPA) 25 mg qd as add-on to metformin for 104 weeks led to significant

and sustained reductions in body weight compared with an increase in body weight with glimepiride (GLIM). Patients had the option to participate in a dedicated body composition sub-study in which we aimed to determine the effects of EMPA vs GLIM on the amount and distribution of body fat.

Materials and methods: Body composition was evaluated in 91 randomised patients in the sub-study. Using whole body dual energy X-ray absorptiometry (DXA), changes from baseline to week 52 and week 104 in trunk fat, limb fat, total fat mass and fat-free mass were assessed in 62 patients (36 receiving EMPA and 26 receiving GLIM). Using magnetic resonance imaging (MRI), changes from baseline to week 52 and week 104 in abdominal visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) were assessed in 51 patients (29 receiving EMPA and 22 receiving GLIM). Changes from baseline were evaluated using mixed model repeated measures (MMRM) analyses.

Results: In the 91 patients evaluated in the sub-study, baseline mean [SD] age was 55.7 [10.4] years, weight was 85.1 [14.7] kg, BMI was 31.9 [4.9] kg/m² and 60% had BMI ≥30 kg/m². EMPA significantly reduced trunk fat, limb fat and total fat mass vs GLIM at week 52 and week 104 (table). EMPA also significantly reduced fat-free mass vs GLIM at week 52 but not at week 104 (table). Adjusted mean (SE) changes from baseline in abdominal VAT at week 52 were -15.5 (5.2) cm² with EMPA compared with +10.0 (6.1) cm² with GLIM ($p<0.01$) and at week 104 were 16.0 (8.4) cm² with EMPA compared with +17.7 (10.0) cm² with GLIM ($p<0.05$). Adjusted mean (SE) changes from baseline in abdominal SAT at week 52 were 29.9 (7.3) cm² with EMPA compared with +25.8 (8.6) cm² with GLIM ($p<0.001$) and at week 104 were 32.5 (9.2) cm² with EMPA compared with +34.4 (10.7) cm² with GLIM ($p<0.001$).

Conclusion: When used as add-on to metformin, treatment with EMPA led to reductions in trunk fat, limb fat, abdominal VAT and SAT at week 52 and further reductions at week 104 compared with GLIM. Reductions in fat-free mass with EMPA vs GLIM were observed at week 52 but not at week 104.

	Baseline			Change from baseline	
	Glimepiride (n=26)	Empagliflozin (n=36)		Glimepiride (n=26)	Empagliflozin (n=36)
Trunk fat, kg	18.9 (1.1)	18.3 (0.8)	Week 52	+1.3 (0.4)	-1.1 (0.3)***
			Week 104	+0.3 (0.5)	-1.9 (0.4)**
Limb fat, kg	13.0 (1.0)	12.0 (0.7)	Week 52	+0.7 (0.2)	-0.8 (0.2)***
			Week 104	-0.1 (0.3)	-1.6 (0.2)***
Total fat mass, %	37.9 (1.9)	37.3 (1.3)	Week 52	+1.5 (0.5)	-0.8 (0.5)**
			Week 104	-0.1 (0.7)	-2.6 (0.5)**
Fat-free mass, kg	53.3 (2.1)	52.6 (1.8)	Week 52	+0.3 (0.4)	-1.5 (0.3)**
			Week 104	+0.8 (0.6)	-0.3 (0.4)

Baseline values are mean (SE). Changes from baseline are adjusted mean (SE) based on MMRM, with baseline HbA1c and baseline of the endpoint in question as linear covariates, and baseline eGFR, region, treatment, visit and visit by treatment interaction as fixed effects, in patients treated with ≥1 dose of study drug who had a baseline HbA1c value and a baseline and on-treatment DXA scan. Observed cases, values after rescue medication excluded.

** $p<0.01$ for difference vs glimepiride; *** $p<0.001$ for difference vs glimepiride.

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Empagliflozin decreases inflammation and AGE/RAGE markers in the aortic vessel wall of a streptozotocin type 1 diabetic model

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Background and aims: Hyperglycemia and associated glucotoxicity is a major cause of vascular complication in patients with Type 1 and Type 2 diabetes. Pharmacological interventions, aiming to decrease blood glucose via stimulation of glucose entry in the tissues appeared unsuccessful to decrease significantly the cardiovascular complications and associated mortality. SGLT2 inhibitors may interrupt the vicious cycle of maintaining glucotoxicity within the tissue in particular in the vessel walls by excreting glucose out of the body rather than pushing it back into the tissue. Therefore we investigated whether empagliflozin, a potent and selective SGLT2 inhibitor has the potential to decrease the inflammation and AGE/RAGE signalling in aortic vessel wall of streptozotocin (STZ) type 1 animal model.

Materials and methods: Type 1 diabetes in Wistar rats was induced by an intravenous injection of STZ (60mg/kg). One week after injection, empagliflozin was administered via drinking water at two doses (10mg/kg Lo dose,

and 30mg/kg Hi dose) for 8 weeks. Two control groups were run in parallel, non-diabetic animals and diabetic animals treated with solvent in drinking water. At the end of treatment, animals were sacrificed and blood sample and aortic vessel wall collected for analysis. Glucose and methylglyoxal (by HPLC) were determined in blood. In vessel wall, several markers of inflammatory pathway, AGE-positive protein, RAGE expression, were determined by quantitative rtPCR or western blot analysis.

Results: Plasmatic glucose and HbA1c were significantly decreasing in a dose dependant manner with empagliflozin treatments. The expression of the NADPH oxidase isoforms Nox1 and Nox2 as well as the general stress response enzyme heme oxygenase-1 (HO-1) was increased in diabetes and significantly reduced by SGLT2i therapy. Aortic RAGE expression and levels of AGE-positive proteins was increased in the STZ group and normalized by SGLT2i therapy. In line with this, serum levels of the AGE precursor methylglyoxal were significantly increased in the STZ group and improved by SGLT2i therapy. The inflammatory genes monocyte chemoattractant protein-1 (MCP-1, CCL-2), the monocyte/macrophage-specific protein CD68 and the cytokine interleukin-6 (IL-6) were up-regulated at the mRNA level in the aorta and significantly reduced by both SGLT2i doses. Likewise the immune-signaling proteins interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α) and the general markers of inflammation, cyclooxygenase-2 (COX-2) or intercellular adhesion molecule-1 (ICAM-1) were all up-regulated in STZ-treated rats and reduced by SGLT2i therapy.

Conclusion: These results show that the specific SGLT2 inhibitor, empagliflozin, by decreasing blood glucose via its excretion in urine is able to decrease several markers of oxidative stress, inflammation and AGE/RAGE signaling in aortic vessel wall of Type 1 diabetic animals. Therefore these results support the improvement of endothelial function that we have observed with empagliflozin in this model and are very promising in term of long term effects of empagliflozin on cardiovascular complications. Ongoing cardiovascular trials in Type 2 diabetic population will address this question.

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Predicted temporal changes in energy intake and energy expenditure in subjects with type 2 diabetes treated with canagliflozin

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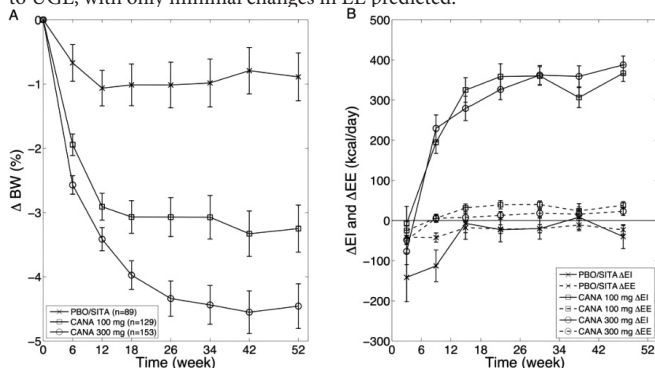
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Background and aims: Canagliflozin (CANA), an SGLT2 inhibitor approved for the treatment of type 2 diabetes, reduces plasma glucose concentrations by increasing urinary glucose excretion (UGE), with sustained increases in UGE of ~80–120 g/day (~320–480 kcal/day) observed with CANA treatment. CANA treatment also induces weight loss, with body weight (BW) decreasing for approximately 26 weeks before reaching a new, lower steady-state BW that is maintained with sustained CANA treatment. Data from animal studies and small short-term studies in humans suggest that energy intake (EI) is increased in response to SGLT2 inhibition. The aim of this analysis was to calculate the temporal changes in energy expenditure (EE) and EI that occur with CANA treatment.

Materials and methods: Data from a 52-week Phase 3 study comparing the efficacy of placebo (PBO), CANA 100 mg, and CANA 300 mg were used in the analysis (N = 371, mean (SD) baseline BW = 87 (21) kg, HbA1c = 7.8 (0.8)%, 55% female). Following the 26-week core study period, PBO-treated subjects were switched to sitagliptin (SITA) 100 mg (which is BW neutral) for the remaining 26 weeks. BW was measured approximately every 6 weeks and treatment-induced UGE increases of 0, 80, and 90 g/day were assumed for PBO/SITA, CANA 100 mg, and CANA 300 mg, respectively, based on data from previous studies. A previously validated mathematical model was used to calculate the changes in EE and EI in each treatment group based on the measured BW and UGE data.

Results: CANA treatment dose-dependently reduced BW (Figure A), with mean (SE) reductions in BW of 0.9 (0.4)%, 3.3 (0.4)%, and 4.5 (0.3)% observed at Week 52 for PBO/SITA, CANA 100 mg, and CANA 300 mg, respectively ($p<0.001$ for both CANA doses vs. PBO/SITA). EI increased over time in CANA-treated subjects until approximately Week 26 when the sustained increase in daily EI nearly matched the daily caloric loss due to UGE (Figure B), thereby obtaining a new state of energy balance and a stable BW. Only small changes in EE were predicted, as the decrease in metabolic rate associated with weight-loss was nearly balanced by increased dietary thermogenesis arising from increased EI (Figure B).

Conclusion: CANA treatment reduces BW due to UGE-associated caloric loss. The sustained UGE increase leads to a period of weight loss followed by a plateau in which a lower equilibrium BW is maintained. The plateau in BW is predicted to be due to sustained increases in EI that match the caloric loss to UGE, with only minimal changes in EE predicted.



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The SGLT2 inhibitor empagliflozin ameliorates early features of diabetic nephropathy in BTBR ob/ob type 2 diabetic mice with and without hypertension

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Background and aims: Diabetic nephropathy is the leading cause of end-stage renal disease in man in the western world. Recent development of sodium-glucose co-transporter 2 (SGLT2) inhibitors offers a new anti-diabetic therapy via enhanced glucose excretion. Whether this strategy exerts beneficial effects on developing type 2 diabetic nephropathy is unclear. We investigated the effects of the SGLT2 inhibitor empagliflozin in BTBR ob/ob mice, which spontaneously develop type 2 diabetic nephropathy.

Materials and methods: In a first experiment, BTBR ob/ob mice either received a diet containing 300ppm empagliflozin or equicaloric placebo chow for 12 weeks. In a second experimental setting, BTBR ob/ob mice received angiotensin (Ang) II and were separated in the same 2 diet groups for 6 weeks. Urine, blood, and tissues were harvested and blood pressure was monitored by tail cuff measurements.

Results: In both experiments, empagliflozin treatment enhanced glucosuria, (6,987±987μmol/mg Crea vs 2,182±972μmol/mg Crea; $P<0.001$; Ang II: 9,716±658μg/mg Crea vs 5,303±947μg/mg Crea; $P<0.05$) thereby lowering blood glucose (204±16 vs 402±27mg/dl $P<0.001$; Ang II: 170±20 vs 410±39mg/dl $P<0.01$). While Ang II infusion induced profound hypertension (146±4 vs 84±1mmHg; $P<0.0001$), empagliflozin treatment had no significant effect on blood pressure in normotensive or hypertensive mice (81±1 vs 84±1mmHg; 139±6 vs 146±4mmHg). In both experiments, empagliflozin reduced albuminuria in diabetic mice (2,291±524 vs 834±135μg/mg Crea, $P<0.001$; Ang II: 4,869±1776 vs 1,470±226μg/mg Crea, $P<0.05$). Empagliflozin treatment did not affect matrix expansion as evaluated by immunohistochemistry, while it decreased the diabetes-related glomerular hypertrophy in normotensive BTBR ob/ob mice (3,392±100.9 vs 3,646±59.8 μm², $P<0.05$), but not in hypertensive mice (3,786±230.0 vs 3,877±88.6 μm²). After 6 weeks, diabetic BTBR ob/ob mice on the placebo diet showed a significant increased MCP-1 (~1.4-fold; $P<0.05$ vs WT), RANTES (~2.6-fold; $P<0.05$ vs WT), and IL-6 (~2.4-fold; $P<0.05$ vs WT) mRNA expression. While none of these inflammatory markers were significantly affected after 6 weeks of empagliflozin treatment, 12 weeks of empagliflozin treatment reduced renal mRNA expression of MCP-1 by ~75% ($P<0.05$ vs placebo), of RANTES by ~55% ($P<0.05$ vs placebo), and of IL-6 by 52% ($P<0.05$ vs placebo).

Conclusion: Taken together, our present results confirm that SGLT2 inhibition by empagliflozin is a good therapeutic option to lower blood glucose levels and improve albuminuria in a type 2 diabetes model without or with hypertension. Moreover, empagliflozin treatment differentially ameliorates markers of renal injury such as glomerular hypertrophy in murine diabetic nephropathy without hypertension. In conclusion these results support the concept of SGLT2 inhibition for the prevention of diabetic nephropathy.

Supported by: Boehringer Ingelheim Pharma GmbH & Co.KG

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Dapagliflozin lowers blood pressure in hypertensive and nonhypertensive patients with type 2 diabetes

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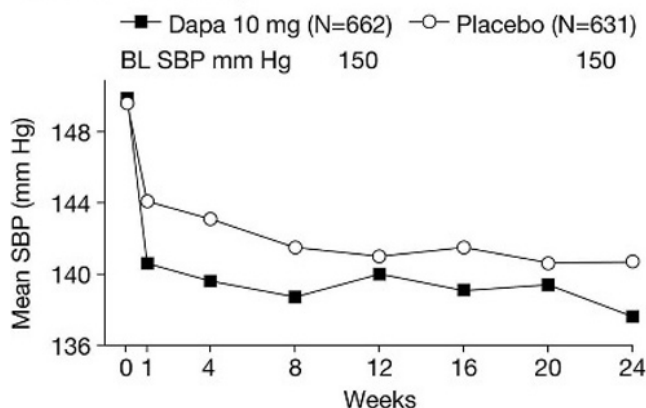
Background and aims: Consistent with mild diuresis and weight loss, dapagliflozin (DAPA), a highly selective sodium-glucose co-transporter 2 inhibitor, also reduces BP in patients (pts) with type 2 diabetes mellitus (T2DM). High BP is associated with an increased risk of cardiovascular events and reducing BP has been shown to reduce this risk regardless of pretreatment BP. Therefore, it is of interest to investigate the ability of DAPA to safely reduce BP across different BP categories in the T2DM population. These analyses evaluated the effect of DAPA on BP in hypertensive (baseline [BL] systolic BP [SBP] > 140) and nonhypertensive (BL SBP ≤ 140) pts across the phase 2b/3 clinical development programme.

Materials and methods: Safety data were pooled from 13 placebo (PBO)-controlled studies in which pts with T2DM were randomised to DAPA 10 mg/d (N=2360) or PBO (N=2295) for up to 24 weeks.

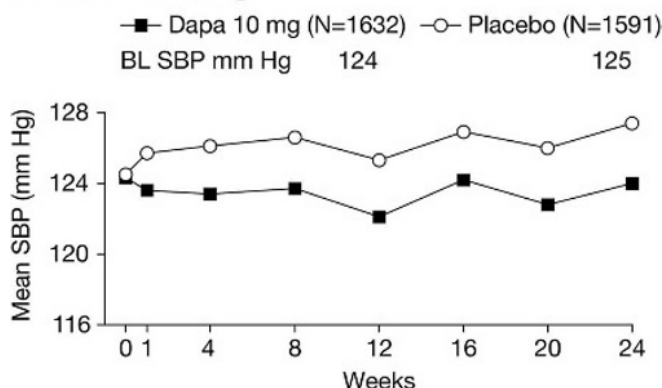
Results: At BL, 91.5% and 91.1% of the hypertensive pts were being treated with antihypertensive medications in the DAPA and PBO groups, respectively, compared with 68.2% and 72.6% of the pts in the nonhypertensive group. In studies with 24 weeks of follow-up, treatment with DAPA resulted in mean PBO subtracted changes (95% CI) in SBP of -3.6 (-4.9 to -2.3) mm Hg in hypertensive, and -2.6 (-3.4 to -1.8) mm Hg in nonhypertensive pts (figure). The corresponding data for diastolic BP (DBP) were -1.2 (-2.0 to -0.4) mm Hg and -1.2 (-1.7 to -0.7) mm Hg, respectively. The difference from PBO in proportion of pts with any measured orthostatic reaction (defined as a decrease from supine to standing of > 20 mm Hg in SBP or > 10 mm Hg in DBP) over 24 weeks was similar in hypertensive (DAPA, 17.4%; PBO, 15.5%), and nonhypertensive (DAPA, 11.4%; PBO, 9.6%) pts. At the 24-week visit there was no increase in the proportion of pts with measured orthostatic reactions in DAPA-treated vs PBO-treated pts in either hypertensive (6.1% vs 6.6%) or nonhypertensive (4.0% vs 4.2%) pts. Adverse events registered under the preferred term "orthostatic hypotension" were rare and none were classified as serious. The highest rate (0.4%) was registered in the nonhypertensive PBO group. There was no clinically significant mean change in heart rate with DAPA in either hypertensive or nonhypertensive pts (-0.5 and 0.1 beats/min, respectively). The proportion of pts on antihypertensive drugs was stable over the observation period (change ≤ 1%).

Conclusion: DAPA 10 mg induces a modest BP reduction across BP categories and may thereby contribute to a reduced cardiovascular risk in hypertensive as well as nonhypertensive T2DM pts. The potential of DAPA to reduce cardiovascular events is currently under investigation in a large-scale outcomes study, DECLARE.

BL SBP > 140 mm Hg



BL SBP ≤ 140 mm Hg



Clinical Trial Registration Number: NCT00263276, NCT00357370, NCT00528372, NCT00528879, NCT00683878, NCT00859898, NCT00680745, NCT00972244, NCT00673231, NCT00984867, NCT00855166, NCT01031680, NCT01042977

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Sodium glucose cotransporter 2 inhibition with empagliflozin reduces microalbuminuria in patients with type 2 diabetes

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Background and aims: Empagliflozin (EMPA) is a sodium glucose cotransporter 2 (SGLT2) inhibitor that lowers HbA1c, blood pressure (BP) and weight in patients with type 2 diabetes (T2D) and has similar potential in type 1 diabetes (T1D). It has also been shown that EMPA reduces renal hyperfiltration associated with T1D, suggesting a renoprotective reduction in intraglomerular pressure. Based on preclinical data, we hypothesised that the addition of EMPA to standard care, including stable doses of renin angiotensin system inhibitors (RASi), would reduce microalbuminuria versus placebo, consistent with attenuated renal injury.

Materials and methods: This post-hoc analysis assessed the effect of EMPA on urine albumin to creatinine ratio (UACR). Data were pooled from 458 patients with T2D and pre-existing microalbuminuria (UACR 30–300 mg/g; mean [SD] estimated glomerular filtration rate [eGFR; modification of diet in renal disease] 87.9 [23.3] mL/min/1.73m²; mean [SD] age 56.7 [10.1] years) who had participated in 1 of 4 Phase III randomised placebo-controlled tri-

als. Changes in UACR were assessed in 438 patients who received EMPA 10 mg (n=141), EMPA 25 mg (n=150) or placebo (n=147) for 24 weeks.

Results: After controlling for baseline log-transformed UACR, HbA1c, systolic BP, eGFR, region, trial and treatment, EMPA 10 mg and EMPA 25 mg significantly reduced UACR by 30% and 25%, respectively, compared with placebo at week 24 (Table; p<0.01).

Conclusion: EMPA 10 mg and EMPA 25 mg reduced microalbuminuria by a clinically meaningful amount when used as add-on to standard therapy including stable RASi in patients with T2D. Prospective studies are needed to examine the potential renal protective effects of EMPA in patients with T1D and in patients with T2D.

	Placebo (n=147)	EMPA 10 mg (n=141)	EMPA 25 mg (n=150)
UACR at baseline ^a , mg/g creatinine	68.2 (64.9)	64.6 (69.8)	69.3 (63.3)
UACR at week 24 ^a , mg/g creatinine	53.6 (46.5, 61.7)	37.6 (32.5, 43.4)	40.0 (34.8, 46.0)
Ratio ^b of relative change from baseline at week 24	0.80 (0.69, 0.92)	0.56 (0.48, 0.64)	0.59 (0.52, 0.68)
Ratio ^c of relative change vs. placebo at week 24		0.70*** (0.57, 0.86)	0.75** (0.61, 0.91)

Patients with T2DM and microalbuminuria (UACR 30–300 mg/g). ^agMean (gCV); ^bAdjusted gMean based on ANCOVA with LOCF imputation (95% CI); ^cAdjusted gMean ratio based on ANCOVA with LOCF imputation (95% CI); data following a change in antihypertensive medication or after glycaemic rescue therapy were excluded. ANCOVA, analysis of covariance; gCV, geometric coefficient of variation; LOCF, last observation carried forward; UACR, urine albumin to creatinine ratio. ***p<0.001; **p<0.01.

Supported by: Boehringer Ingelheim and Eli Lilly

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Albuminuria, the sentinel marker of renal failure, is consistently decreased with empagliflozin in several preclinical models of diabetic nephropathy

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Background and aims: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease worldwide. The earliest clinical sign of diabetic nephropathy is microalbuminuria. Moreover albuminuria is considered a predictive surrogate marker for cardiovascular and renal risk. Long term renal and/or cardiovascular protection with the emergent new class of anti-diabetic drugs, SGLT2 inhibitors, are not yet established. However we were interested to determine whether empagliflozin, a potent, selective sodium glucose co-transporter 2 inhibitor, could impact the sentinel marker of renal disease albuminuria in different preclinical models of diabetic nephropathy.

Materials and methods: Type 1 diabetic Akita mice, hypertensive Type 2 diabetic Dahl-STZ rats, normotensive and hypertensive (with angiotensin implant) BTBR ob/ob mice were treated for several weeks (9–15 weeks) with empagliflozin (10 to 30mg/kg). Dahl-rats, made hypertensive with 4% high salt diet and diabetic with an injection of 50mg/kg streptozotocin were also treated with an ACE inhibitor (lisinopril 10mg/kg) or empagliflozin (20mg/kg) in drinking water or with the combination of both drugs. Blood and urine were collected to determine plasmatic glucose concentration and proteinuria respectively. Blood pressure (BP) was determined using the tail-cuff system.

Results: As expected, in all these different preclinical models empagliflozin was effective in lowering significantly blood glucose (–58% up to –100%: normalization). A 76% blood pressure reduction was observed in Akita mice with empagliflozin while no effect on blood pressure was observed in normotensive and hypertensive BTBR ob/ob mice. In hypertensive Dahl-STZ rat, either lisinopril or empagliflozin was able to reduce blood pressure while the combination induced a significant drop in blood pressure. Interestingly, proteinuria was significantly and consistently reduced by empagliflozin in diabetic mice (~ –60 %). In Dahl-STZ rat, lisinopril alone did not reduce proteinuria while empagliflozin decreased it by ~28%. When used in combination with lisinopril the effect of empagliflozin on proteinuria was even further pronounced (~–50%).

Conclusion: These results show that the specific SGLT2 inhibitor, empagliflozin, is able - on top of lowering blood glucose levels - to consistently decrease proteinuria, in several preclinical models of nephropathy independent of their origin as Type 1 or type 2 diabetes and also independent of their BP status. Whereas these are promising results hinting towards a renal benefit

on the long term use of empagliflozin, it is premature to speculate about the clinical outcomes with empagliflozin. Studies are currently underway to address this question.

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Dapagliflozin lowered ambulatory BP in patients with type 2 diabetes mellitus and hypertension inadequately controlled by a renin-angiotensin system blocker \pm another agent

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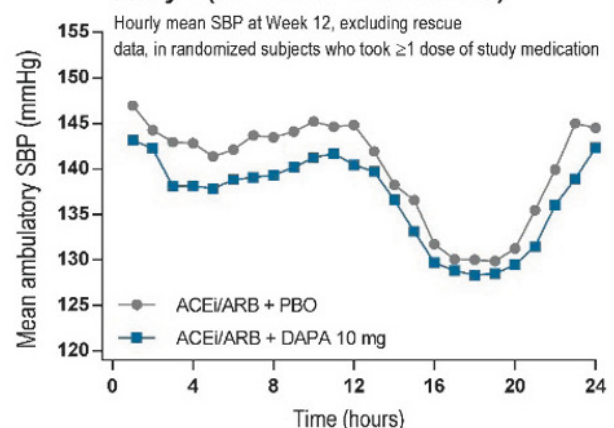
Background and aims: Hypertension is common in patients with type 2 diabetes mellitus (T2DM) and treatment usually starts with an ACE inhibitor (ACEi) or an angiotensin receptor blocker (ARB), with other antihypertensive (AHT) therapies added if required. Dapagliflozin reduces renal glucose reabsorption accompanied by weight reduction and a diuretic effect, both contributing to BP reduction. Here we assess the effects of dapagliflozin in two studies of patients with inadequately controlled T2DM and hypertension.

Materials and methods: Two randomized, double-blind studies assessed the effects of 12 weeks of dapagliflozin 10 mg or placebo on BP in patients with inadequately controlled T2DM (HbA1C 7.0–10.5%) and hypertension (seated systolic BP [SBP] / diastolic BP: 140–164 / 85–104 mmHg) despite receiving glucose-lowering drugs and an ACEi or ARB (Study 1) plus a 2nd AHT agent (Study 2). Preliminary results have been presented previously; here we describe ambulatory BP data.

Results: In Study 1, 613 patients on an ACEi or ARB were randomized to dapagliflozin or placebo. At Week 12, mean 24-hour ambulatory SBP was 2.9 (SEM: 1.01) mmHg lower with dapagliflozin versus placebo ($p=0.0043$, Figure) and night-time SBP was reduced versus placebo (-2.34 [1.16] mmHg). In Study 2, 449 patients on an ACEi or ARB plus another AHT drug (thiazide/thiazide-like diuretics in ~44% of patients, calcium channel blockers ~27%, beta blockers ~27%) were randomized to dapagliflozin or placebo. At Week 12, mean 24-hour ambulatory SBP was lower with dapagliflozin versus placebo (-4.5 [1.37] mmHg, $p=0.0012$, Figure) and a reduction in night time SBP was also evident versus placebo (-3.9 [1.51] mmHg). Dapagliflozin was well tolerated and heart rate showed no significant change from baseline.

Conclusion: Dapagliflozin significantly reduced mean 24-hour ambulatory SBP versus placebo in patients with T2DM and hypertension in combination with an ACEi or ARB \pm 1 AHT over 12 weeks.

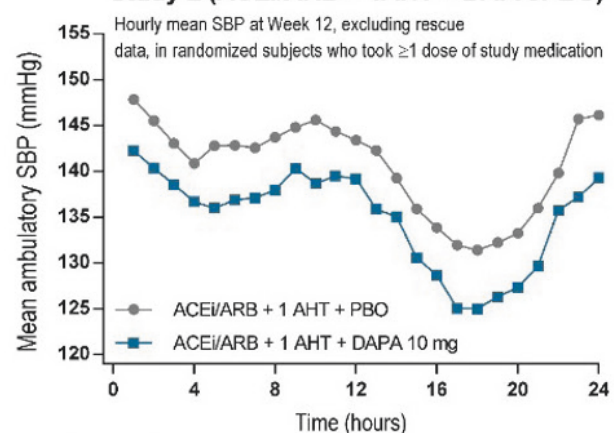
Study 1 (ACEi/ARB + DAPA/PBO)



LOCF sample size at time point

PBO	263	252	263	263	260	261	231
DAPA 10 mg	267	260	264	266	263	263	225

Study 2 (ACEi/ARB + 1AHT + DAPA/PBO)



LOCF sample size at time point

PBO	186	178	184	184	183	184	151
DAPA 10 mg	187	181	184	185	184	185	158

AHT, antihypertensive; ARB, angiotensin receptor blocker; DAPA, dapagliflozin; LOCF, last observation carried forward; PBO, placebo; SBP, systolic BP

Clinical Trial Registration Number: NCT01137474, NCT01195662

Supported by: BMS and AstraZeneca

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Impact of weight loss on weight-related quality of life (WRQL) and health satisfaction (HS): evidence from a head-to-head study of canagliflozin (CANA) vs sitagliptin (SITA)S. Traina¹, A. Slee², C. Neslusan¹;¹Janssen Global Services, LLC, Raritan, ²Axio, Seattle, USA.

Background and aims: The positive health impacts of performing diabetes self-care (e.g., healthy food choices, increased physical activity, and medication taking) are not always apparent to a person with type 2 diabetes mellitus (T2DM); therefore, adherence can be difficult. People with T2DM are more likely to adhere to treatments that offer noticeable benefits, such as convenience, avoidance of hypoglycemic episodes, and weight loss vs. those that do not. Previous studies have demonstrated associations between WRQL and disease outcomes and between HS and performance of self-care behaviors. In this study, we explore the impact of the amount of weight loss demonstrated with CANA treatment on WRQL as measured by the Impact of Weight on Quality of Life (IWQOL-Lite) and physical HS (which includes satisfaction with weight, energy, appetite, sleep, physical activity, and general health) as measured by the Current Health Satisfaction Questionnaire (CHES-Q). In a previously reported study of CANA vs. SITA in dual therapy with metformin and background diet and exercise, CANA was associated with a mean weight loss of -2.1 kg to -2.5 kg vs. SITA after 52 weeks.

Materials and methods: In that study, the IWQOL-Lite and CHES-Q were administered at baseline and over time. Post hoc analyses were used to explore the strength of the relationship between weight change and improvement in IWQOL-Lite total and physical HS scores. Empirical distributions of scores were examined to investigate floor and ceiling effects. The final analytical sample was limited to subjects with baseline scores that were not at the ceiling (e.g., allowing for improvement in score). Differences in least squares mean (LSM) change in weight by changes in IWQOL-Lite and CHES-Q after 52 weeks were compared using ANCOVA models adjusting for selected baseline covariates (i.e., weight, age, and gender). Logistic regression, adjusted for the same covariates, was used to generate odds ratios (ORs) for score changes associated with weight change.

Results: Empirical distributions of change scores indicated that 29.1% of IWQOL-Lite and 5.4% of CHES-Q scores were at the ceiling at baseline; these scores did not decline over time and were thus excluded from the analytical sample. Subjects with improved IWQOL-Lite total and CHES-Q physical scores lost more weight than those with no score improvement (IWQOL-Lite: -3.54 kg; 95% CI -4.07, -3.01 and -2.33 kg; 95% CI -2.78, -1.87, respectively; LSM difference -1.21 kg; 95% CI -1.92, -0.51; CHES-Q: -3.50 kg; 95% CI -3.89, -3.12 and -1.86 kg; 95% CI -2.25, -1.47, respectively; LSM difference -1.65 kg; 95% CI -2.17, -1.12). Weight loss of 2 kg was associated with a 68% greater chance of IWQOL-Lite total score improvement (OR 1.68; 95% CI 1.16, 2.42) and a 72% greater chance of CHES-Q physical domain score improvement (OR 1.72; 95% CI 1.28, 2.31).

Conclusion: Results suggest that the magnitude of weight loss shown in clinical trials to be associated with CANA vs. SITA is noticeable by and important to people living with T2DM. Treatments providing adequate glycemic control that also offer a weight loss benefit are likely to improve WRQL and HS, and in turn, may support persistent and consistent performance of healthy behaviors.

Clinical Trial Registration Number: NCT01106677

Supported by: Janssen Research & Development, LLC

PS 063 GLP-1 based therapies: efficacy I

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The 2 week fasting glucose as a predictor of response to once weekly dulaglutide 1.5 mgT. Forst¹, S. Gough², G. Grunberger³, V. Pechtn⁴, R. Shaginan⁵, H. Wang⁶, L. Fernandez⁶;¹Profil Mainz, Germany, ²University of Oxford and Oxford University Hospitals NHS Trust, UK, ³Grunberger Diabetes Institute, Bloomfield Hills, USA, ⁴Lilly Diabetes, Eli Lilly and Company, Neuilly-Sur-Seine, France,⁵Lilly Diabetes, Eli Lilly and Company, Houten, Netherlands,⁶Lilly Diabetes, Indianapolis, USA.

Background and aims: The identification of patients most likely to respond to a specific treatment is important when attempting to individualize patient care. Predictors of treatment success for weekly GLP-1 receptor agonists are, to date, largely unknown. This analysis was therefore conducted to assess whether laboratory fasting blood glucose (FBG) in patients with type 2 diabetes mellitus (T2DM) measured early in treatment with the once weekly GLP-1 receptor agonist dulaglutide (DU) 1.5 mg predicts treatment response.

Materials and methods: Post hoc analyses were conducted separately for 2 double-blind, randomized Phase 3 studies (AWARD-5, in combination with metformin, and AWARD-1, in combination with metformin and pioglitazone) in patients with T2DM assigned to once weekly DU 1.5 mg. Baseline tertile values from AWARD 5 were used to categorize FBG at baseline and weeks 2 and 8 as follows: Low (L, <7.9 mmol/l); Intermediate (I, ≥7.9 to <10.3 mmol/l); and High (H, ≥10.3 mmol/l). Treatment response in AWARD-5 was assessed at week 12 and 26 by the following composite efficacy endpoint (CEE): HbA_{1c} <7.0% (53 mmol/mol) or HbA_{1c} reduction from baseline >0.8% [if baseline HbA_{1c} <8.0% (64 mmol/mol)]; >1.1% [if baseline A1c ≥8.0% (64 mmol/mol) and <9.0% (75 mmol/mol)]; or >1.6% [if baseline HbA_{1c} ≥9.0% (75 mmol/mol)]. The association between FBG categories and the CEE was analyzed using chi-square tests. The association between week 2 FBG and HbA_{1c} treatment response at weeks 13 and 26 was validated in AWARD-1 using the same FBG categorization and CEE definition.

Results: In AWARD-5, mean baseline HbA_{1c} for DU 1.5 mg (N=304) was 8.1% (65 mmol/mol). At baseline, mean FBG was 9.8 mmol/l and 33% (n=99), 32% (n=97), and 36% (n=108) of patients had FBG in the L, I, and H categories, respectively. After 2 weeks of treatment, mean FBG was 7.2 mmol/l and 68% (n=208), 21% (n=64), and 11% (n=32) of patients had FBG in L, I, and H categories, respectively. At week 26, mean HbA_{1c} was 6.9% (52 mmol/mol). There was a strong association between FBG at week 2 and achieving the CEE at week 26 ($p<0.001$). A significantly higher percentage of patients in FBG category L (83% [172/208]) at week 2 met the CEE at week 26 compared to patients in FBG categories I (61% [39/64]), $p<0.001$, and H (34% [11/32]), $p<0.001$, at this time. CEE results at week 12 were consistent with those at week 26. Similar findings were seen using AWARD-1 data.

Conclusion: In patients treated with once weekly DU 1.5 mg in AWARD-5 and AWARD-1, FBG values at week 2 were strongly associated with treatment response at week 26 as measured by a CEE. The probability of achieving a treatment response at week 26 was greatest for patients in the lower FBG categories at week 2. FBG values at week 2 may be an early and useful measurement for predicting response to once weekly DU 1.5 mg treatment in patients with T2DM.

Supported by: Eli Lilly and Company

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Real-world comparative effectiveness of exenatide once weekly and liraglutide in patients with type 2 diabetes mellitusW. Saunders¹, H. Hiep Nguyen², I. Kalsekar²;¹Saunders Research, Charlotte, ²AstraZeneca, Fort Washington, USA.

Background and aims: Exenatide once weekly (EQW) and liraglutide once daily (LIRA), glucagon-like peptide-1 receptor agonists (GLP-1RAs), have demonstrated improvements in glycaemic outcomes in patients with type 2 diabetes mellitus (T2DM) in randomized clinical trials. However, little is known about their real-world comparative effectiveness. This retrospective cohort study used the Quintiles Electronic Medical Record (EMR) database

to evaluate the 6-month change in HbA_{1c} for patients initiating EQW or LIRA.

Materials and methods: Patients with T2DM prescribed EQW or LIRA between 01 February 2012 and 31 May 2013 were identified. Baseline HbA_{1c} measures were from 75 days prior to 15 days after initiating EQW or LIRA, with follow-up measures documented at 6 months (± 45 days). Adjusted linear regression models compared the difference in mean HbA_{1c} change. *A priori* defined sensitivity analyses were performed in the subgroup of patients with baseline HbA_{1c} $\geq 7.0\%$ and no prescription for insulin during the 12-month pre-index period.

Results: For EQW and LIRA respectively, mean (SD) age of the main study cohort was 58.0 (11.0) and 58.1 (11.0) years, mean (SD) baseline HbA_{1c} was 8.3% (1.7) and 8.4% (1.6), and 48.1% and 54.1% of patients were women. In adjusted models, change in HbA_{1c} did not differ between EQW and LIRA during 6 months of follow-up (Table). Results were consistent in the subgroup analyses.

Conclusion: In a real-world setting, HbA_{1c} similarly improved in patients initiating EQW or LIRA.

Change in HbA _{1c} from Baseline at 6 Months					
Data are mean (SD)					
Population	Cohort	Unadjusted change	P value	Adjusted change**	P value
All patients	Exenatide QW (n=644)	-0.60 (1.50)	0.2997	-0.64 (1.32)	0.8722
	Liraglutide (n=3184)	-0.66 (1.52)		-0.65 (1.31)	
Subgroup*	Exenatide QW (n=294)	-0.83 (1.54)	0.3442	-0.87 (1.29)	0.5589
	Liraglutide (n=1419)	-0.93 (1.55)		-0.92 (1.28)	

*Patients not at goal (HbA_{1c} $\geq 7\%$) at baseline and had no pre-index insulin use. **Adjusted for age, gender, race, baseline HbA_{1c}, BMI, number of other antidiabetes medication classes, Deyo-Charlson Comorbidity Index, insurance type, region, hyperlipidemia, and hypertension.

Supported by: Bristol-Myers Squibb/AstraZeneca

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Effectiveness of lixisenatide before breakfast or the main meal using CGM with AGP analysis

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Background and aims: Lixisenatide is a once-daily glucagon-like peptide-1 receptor agonist that has been shown by 7-point self-monitoring blood glucose to lower postprandial and fasting glycaemia. The aim of this study was to understand, in a subset of type 2 diabetes mellitus (T2DM) patients in a multicentre randomized trial, the change in diurnal glucose patterns using continuous glucose monitoring (CGM) prior to and during lixisenatide treatment based on the time of administration: before breakfast (BK) or the main meal (MM) as defined by the patient.

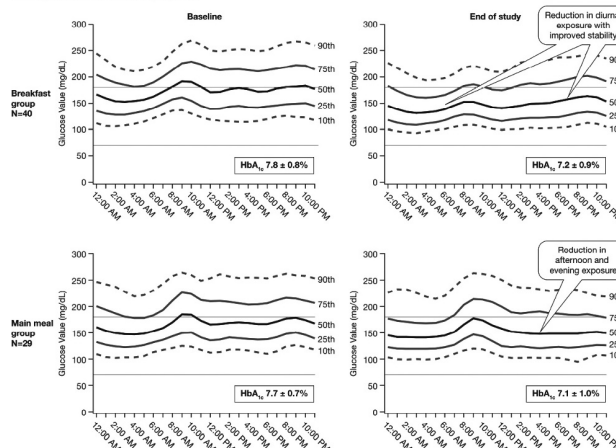
Materials and methods: Blinded CGM was used 14 days before and during lixisenatide treatment (BK or MM). CGM data were analyzed using Ambulatory Glucose Profile (AGP) analysis to detect changes in diurnal glucose patterns.

Results: Sixty-nine patients completed (T2DM 7.8 \pm 4.9y; age 57.8 \pm 10.2y; 53.6% female). AGP analysis showed significant reduction in 24h glucose exposure (AUC) in both groups (BK: 4918.1 \pm 652.3 to 3681.2 \pm 699.6 mg/dL*24h, $p<0.0001$; MM: 4127.9 \pm 876.6 to 3880.9 \pm 1165.0 mg/dL*24h, $p=0.0224$). Reduction in hourly waking AUC was significant in both groups

(BK: 181.1 \pm 28.1 to 157.2 \pm 29.5 mg/dL*h, $p<0.0001$, MM: 177.9 \pm 37.7 to 165.6 \pm 48.2 mg/dL*h, $p=0.0184$). However, hourly sleeping AUC reduction was significant in the BK group (161.8 \pm 28.0 to 144.6 \pm 28.8 mg/dL*h, $p<0.001$) and not in the MM group (159.8 \pm 36.4 to 153.4 \pm 51.5, $p=0.0942$). Variability (IQR) improved in both groups, although was not significant. Subjects in both groups had less than 1% of time spent in hypoglycaemic range (<60 mg/dL). HbA_{1c} reduction was similar in both groups. Figure 1 shows composite AGPs for each group.

Conclusion: In summary, lixisenatide before MM appears to benefit afternoon and evening glucose exposure, while lixisenatide before BK appears to have a sustained improvement in the overall diurnal glucose patterns.

Figure 1. Composite AGPs for the BK and MM groups at baseline and study end. The AGP diurnal glucose profiles indicate that patients in the BK had improvements in sleeping, waking, post-meal glucose exposure and stability, whereas those in the MM group shows reductions in HbA_{1c}, primarily due to lowering of afternoon and evening exposure.



Clinical Trial Registration Number: NCT01517412
Supported by: Sanofi

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Harmony 2 year 3 Results: albiglutide monotherapy in drug naïve patients with type 2 diabetes mellitus

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Background and aims: This 3 year (y), double-blind, placebo (Pbo)-controlled study examined efficacy/safety of albiglutide at 30mg (A30) and 50mg (A50) vs Pbo in patients with A1C (53–85.8 mmol/mol [7–10%]) on diet and exercise.

Materials and methods: Both Albi arms began A30 QW (A50 started at Wk 12) and continued on randomized medication after hyperglycemic rescue. Primary endpoint (PE) was A1C change from baseline at Wk 52.

Results: Baseline characteristics were similar between arms; mean A1C 65 mmol/mol (8.1%); age 53 y; diabetes duration 4 y. The PE reported previously showed Wk 52 A1C difference (Albi – Pbo) -9.2 mmol/mol (95% CI: -12.1, -6.3) [-0.84% (95% CI: -1.11, -0.58)] for A30 and -11.4 mmol/mol (95% CI: -14.3, -8.4) [-1.04% (95% CI: -1.31, -0.77)] for A50; both $P<0.0001$. In pts completing 3y without rescue, A1C reduction was durable to Wk 156 in both albi arms (-10.5 mmol/mol, SD 10.6 [-0.96%, SD 0.968], A30 (n=30), -11.7 mmol/mol, SD 9.7 [-1.07%, SD 0.887], A50 (n=32), -6.7 mmol/mol, SD 7.0 [-0.61%, SD 0.644], Pbo (n=14), with generally greater reductions in the A50 arm. FPG (mmol/L [mg/dL]) decreased in a dose dependent manner in the albi arms, (-1.3, SD 1.77, A30; -1.8, SD 2.01, A50; -0.23, SD 0.81, Pbo [-23.6, SD 31.87, A30; -32.9, SD 36.14, A50; -4.3, SD 14.63, Pbo]) and was maintained to Wk 156. Modest weight loss (kg) occurred in all arms (-2.91 Pbo, -1.32 A30, -2.24 A50). The probability of requiring rescue by Wk 156 was 74% Pbo, 54% A30, and 42% A50. Metformin was the most common rescue medication. In pts completing 3y with rescue, A1C reduction was -7.5 mmol/mol, SD 14.1 [-0.69%, SD 1.293], A30 (n=66), -9.3 mmol/mol, SD 9.3 [-0.85%, SD 0.85], A50 (n=51), -9.0 mmol/mol, SD 14.4 [-0.82%, SD 1.319], Pbo (n=47), confounded by a higher use of rescue medication in the Pbo arm. On-therapy adverse event (AE) % was higher in the A30 and A50 arms than Pbo 89.1/89.9/83.1 but SAE % was similar 14.9/17.2/15.8. GI AEs for Pbo/A30/A50 were: nausea 10/14/11; diarrhea 15/13/16; vomiting 1/4/4. Injection site reactions were higher for A30 and A50 vs Pbo 22/28/11. Pre-rescue

documented (≤ 3.88 mmol/L [70 mg/dL]) symptomatic hypoglycemia events were low: A30 2%, A50 1%, Pbo 3%; no severe events reported.

Conclusion: Albi monotherapy resulted in robust, durable A1C reduction through Wk 156 and was generally well tolerated.

Clinical Trial Registration Number: NCT00849017

Supported by: GSK

831

Harmony 3 year 3 Results: albiglutide vs sitagliptin and glimepiride in patients with type 2 diabetes mellitus on metformin

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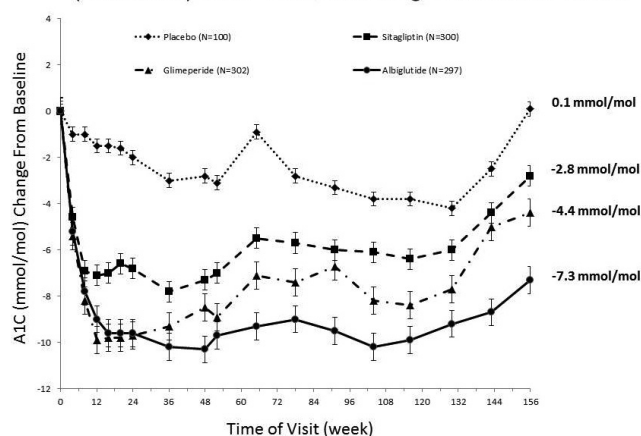
Background and aims: This 3-year (y), randomized (rzd), double-blind, placebo (Pbo) controlled, study examined albiglutide (Albi) 30mg vs placebo (Pbo), sitagliptin (Sita), or glimepiride (SU) in patients (pts) with T2DM (A1C 53–85.8 mmol/mol [7–10%]) on Metformin (Met).

Materials and methods: Pts meeting predefined hyperglycemia criteria qualified for blinded dose titration (SU 2–4mg, Albi 30–50mg). Pts received hyperglycemic rescue if prespecified rescue criteria were met & continued receiving rzd medication. Pts (%) who completed the 3y study (including rescue): Pbo 53, Sita 61, SU 60 & Albi 61.

Results: Primary endpoint at Wk 104 (baseline A1c 65 ± 8.7 mmol/mol [$8.1 \pm .8\%$]), Albi treatment difference was superior to Pbo (-9.9 mmol/mol [$-.91\%$]; $P < .0001$), Sita (-3.8 mmol/mol [$-.35\%$]; $P = .0001$) & SU (-3.0 mmol/mol [$-.27\%$]; $P = .003$). At Wk 156, probability of rescue (%) was lower in Albi: Pbo 65.6 ($P < .0001$), Sita 47.8 ($P = .0124$), SU 44.3 ($P = .1605$), Albi 37.3. In pts completing 3y of treatment with & without rescue, durability of Albi was demonstrated with both A1c & FPG reduction from baseline. There was minimal change in weight at 3y (kg): Pbo $-.41$, Sita $-.61$, albi -1.0 & weight gain with SU $+1.60$. GI AEs (% pts) to Wk 156 with Pbo/Sita/SU/Albi were: nausea 13/7/8/12 & vomiting 1/5/4/7. Prerescue documented (≤ 3.88 mmol/L [70 mg/dL]) symptomatic hypoglycemic events (%) were Pbo 4.0, Sita 1.7, SU 19.9, Albi 3.6; with 1 severe event (SU).

Conclusion: We conclude that Albi treatment, as add on to Met, was generally well tolerated with maintained glycemic control over a 3y study period.

Line Graph of Mean (\pm SE) Change From Baseline in A1C (mmol/mol) Over Time, Including Postrescue Values



Clinical Trial Registration Number: NCT00838903

Supported by: Research funded by GSK

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Effectiveness of switching from a DPP-4 inhibitor to the human GLP-1 analogue liraglutide in patients with type 2 diabetes: data from the EVIDENCE study

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Background and aims: We used data from a post-marketing study in France to assess the effectiveness of switching from a DPP-4 inhibitor (DPP-4i) to liraglutide in patients with type 2 diabetes.

Materials and methods: EVIDENCE is a multicentre, observational, post-marketing outpatient study requested by the French National Health Authority to evaluate the efficacy and safety of the human GLP-1 analogue liraglutide. Its primary objective is to determine the percentage of patients still taking liraglutide and at HbA1c target ($< 7\%$) after 2 years. Statistical analyses: for quantitative variables a normality Kolmogorov-Smirnov test was used; comparisons of two dependent groups were performed using the Wilcoxon signed rank test; for paired qualitative variables the McNemar test was used.

Results: Data were collected from 3152 subjects of whom 1261 (40%) were receiving a DPP-4i prior to liraglutide initiation. A total of 1002 (32%) subjects switched from a DPP-4i to liraglutide at the start of the study. Baseline characteristics of subjects who switched and were still on liraglutide treatment at the end of the study are presented in Table 1. These subjects ($n = 624$) achieved significant reductions in mean HbA1c (-0.85% , $p < 0.0001$), fasting plasma glucose (-0.28 g/l, $p < 0.0001$) and body weight (-3.60 kg, $p < 0.0001$). An increased percentage of subjects reached the HbA1c target of $< 7\%$ after switching to liraglutide (31.7% at the end of the study vs. 9.7% at baseline; $p < 0.0001$). Withdrawals (21.4% in overall study cohort) were mostly due to gastrointestinal disorders experienced at the start of the study.

Conclusion: Switching from a DPP-4i to liraglutide led to significant improvements in glycaemic control and body weight in this observational study. In a randomised controlled trial, switching from the DPP-4 inhibitor sitagliptin to liraglutide provided significant HbA1c (-0.5% ; $p < 0.0001$) and body weight reductions (-2.5 kg; $p < 0.0001$). The greater reductions in HbA1c and body weight in this observational study versus the controlled setting of a clinical trial may reflect different baseline characteristics and concomitant diabetes medications. However, these data support the potential benefits of switching from a DPP-4i to a GLP-1 analogue, as previously observed in a randomised clinical trial.

Table: Baseline characteristics of subjects who switched from a DPP-4i to liraglutide and still on liraglutide treatment at the end of the study

Baseline characteristics	
N	624
Age, years	58 ± 10
Diabetes duration, years	9.2 ± 5.8
HbA1c, %	8.4 ± 1.4
Fasting plasma glucose, g/l	1.8 ± 0.6
Body weight, kg	96 ± 18
BMI, kg/m ²	34 ± 6
Proportion of patients with HbA1c $< 7\%$	9.7%

Data are mean \pm SD

Clinical Trial Registration Number: NCT01226966

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Safety and tolerability of liraglutide 3.0 mg in overweight and obese adults: the SCALE obesity and prediabetes randomised trial

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Background and aims: The primary objective of this trial was to investigate the efficacy of the GLP-1 receptor agonist (RA) liraglutide 3.0 mg, as adjunct to diet and exercise, for weight management. Key safety and tolerability data are presented.

Materials and methods: Individuals (BMI ≥ 27 kg/m² with ≥ 1 comorbidity or ≥ 30 kg/m²) were advised on a 500 kcal/day deficit diet and exercise programme and randomised 2:1 to once daily sc liraglutide 3.0 mg (n=2487) or placebo (n=1244). Certain event types (deaths, select CV disorders, pancreatitis, neoplasms, thyroid disorders requiring thyroidectomy) underwent blind independent assessment.

Results: Baseline characteristics: age 45.1 years, 78.5% female, body weight 106.2 kg, BMI 38.3 kg/m². Liraglutide 3.0 mg induced greater weight loss (primary endpoint; 8.0%) vs placebo (2.6%) after 56 weeks (estimated treatment difference [ETD] -5.4%; $p < 0.0001$). Consistent with the known effects of GLP-1RAs, the most common AEs with liraglutide 3.0 mg were mild/moderate nausea and diarrhoea, mostly transient and with onset in weeks 1–4. AEs leading to withdrawal occurred in 9.9% of individuals on liraglutide 3.0 mg, mostly in weeks 1–12 and due to GI AEs, vs 3.8% with placebo. Similar proportions reported serious AEs in both treatment groups (6.3% vs 5.0%). None occurred in $\geq 1\%$ of individuals. 3 deaths occurred: liraglutide 3.0 mg (n=1) vs placebo (n=2) (1 CV-related death in each group). Liraglutide treatment was not associated with increased risk of depression or suicidality. Gallbladder-related AEs were more common with liraglutide 3.0 mg vs placebo (2.5% vs 1.0%), due to more events of ‘cholelithiasis’ and ‘cholecystitis’. The frequency of pancreatitis was higher with liraglutide (0.3%) vs placebo (0.1%). 1 individual per group co-reported gallbladder-related AEs and pancreatitis. An increase in mean serum lipase activity was seen with liraglutide, but few individuals (2.5% vs 1.1% with placebo) had levels ≥ 3 times the UNR. Most elevations were transient and not predictive of pancreatitis. Liraglutide reduced mean SBP and DBP vs placebo at week 56 (ETD -2.8 and -0.9 mmHg, $p < 0.001$), but increased mean pulse (ETD 2.4 bpm, $p < 0.0001$) and a persistent increase of > 20 bpm at ≥ 2 consecutive visits occurred in 5.1% vs 1.4% of individuals, respectively. CV events were similar with liraglutide (8.7%) vs placebo (9.9%). There were no cases of medullary thyroid carcinoma or C-cell hyperplasia and liraglutide did not increase calcitonin. Injection site reaction rates were 22.4 and 14.9 events/100 patient years of exposure (PYE) with liraglutide and placebo, respectively, with similar allergic reaction rates between treatments (2.6 and 3.2 events/100 PYE).

Conclusion: The safety profile of liraglutide 3.0 mg was consistent overall with the known effects of a GLP-1RA. The clinical significance of the imbalance in gallbladder and pancreatitis events is currently unknown.

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Efficacy and safety of liraglutide for perioperative blood sugar control in diabetic subjects within enhanced recovery after surgery protocols

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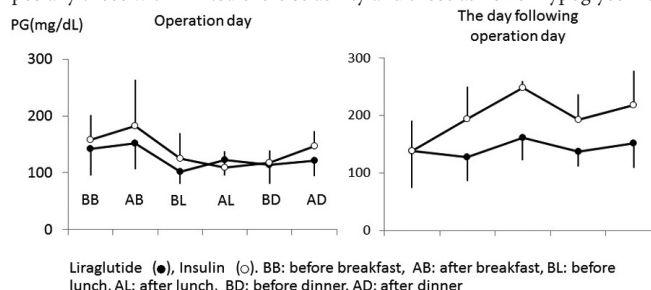
Background and aims: Enhanced recovery after surgery (ERAS) protocols are multimodal perioperative care pathways designed to achieve early recovery after surgical procedures by maintaining preoperative organ function and reducing the profound stress response following surgery. The key principles of the ERAS protocol include perioperative nutritional management such as avoidance of perioperative fasting and carbohydrate loading up to 2 hours preoperatively. This perioperative nutritional management may result in blood sugar fluctuations in insulin-dependent diabetic patients. It has been

controversial to employ glucagon-like peptide-1 receptor (GLP-1R) agonist therapy for perioperative glycemic control. To investigate the efficacy and safety of liraglutide, a GLP-1R agonist, for perioperative glycemic control of surgical patients within ERAS protocol, we performed a randomized prospective study.

Materials and methods: Informed consents were obtained from 25 subjects with T2DM undergoing elective operations within ERAS protocol. Twelve subjects (male; 7, Age; 67.3 ± 8.7 years old) were initiated with liraglutide, and 13 subjects (male; 6, Age; 67.1 ± 9.3 years old) were administered insulin therapy before elective operations, e.g. orthopedic operations (23 cases) and urological surgery (2). In cases of hyperglycemia during perioperative period, regular insulin was added. As GLP-1 decreases gastrointestinal motility, surgical cases with gastrointestinal tracts were excluded. Fluctuation in glycemic level, hypoglycemic events, BMI change, doses of insulin administered if needed and perioperative complications were analyzed.

Results: Liraglutide was continued through the entire period of hospital stay including the operation day (day 0). The mean glucose levels of liraglutide or insulin therapy on day 0 and day 1 were 137.6 ± 26.41 , 138.2 ± 33.1 and 146.5 ± 24.4 , 187.1 ± 35.6 mg/dl, respectively. The body weight of patients receiving liraglutide or insulin therapy before the operation decreased -4.7 ± 1.0 kg or -1.8 ± 0.9 kg, respectively. Additional regular insulin was not needed except in the case of 1 patients. There was an additional benefit of body weight loss enabling efficient rehabilitation. Hypoglycemic events, wound healing retardations, or other complications were not observed in patients receiving liraglutide.

Conclusion: Liraglutide provides an effective and optional way for perioperative blood sugar control within ERAS protocols in patients with T2DM, especially those with limited exercise ability and those at risk of hypoglycemia.



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Novel combination of insulin degludec and liraglutide (IDegLira) is efficacious across the range of disease progression in type 2 diabetes

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Background and aims: This study explored whether a novel combination of insulin degludec (IDeg) and liraglutide (Lira), IDegLira, was consistently effective across three parameters of type 2 diabetes (T2D) progression: HbA_{1c}, diabetes duration and dose of insulin.

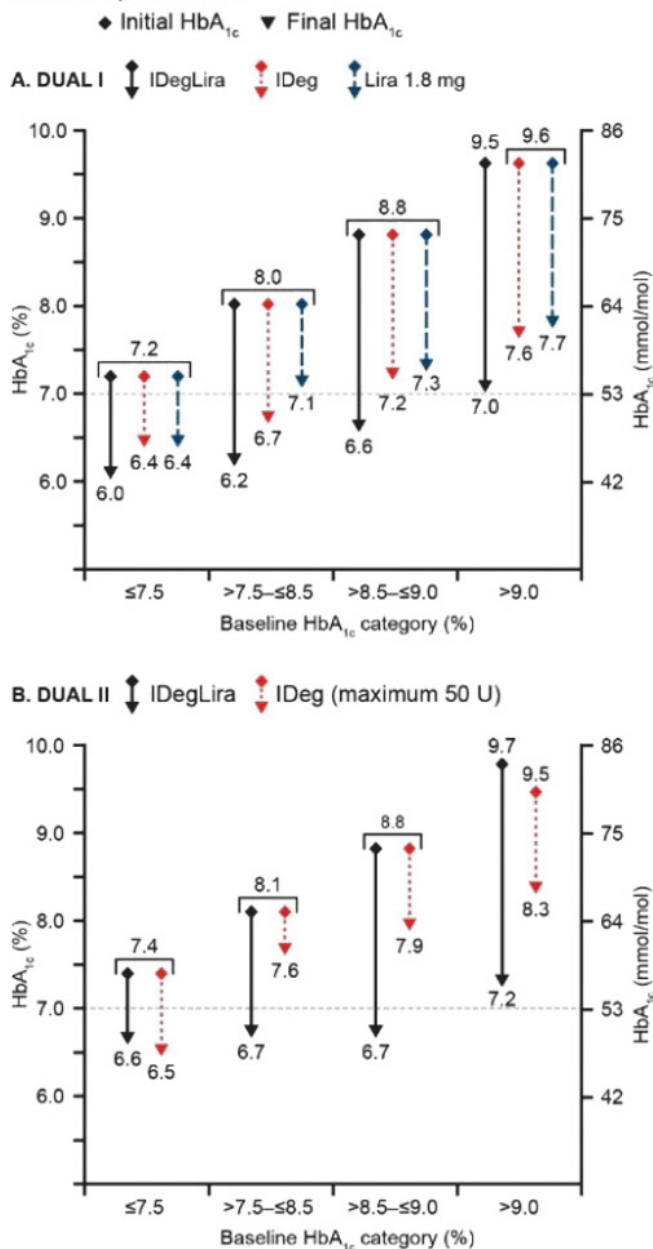
Materials and methods: Mean HbA_{1c} reductions of 1.8 and 1.9% were observed with IDegLira in two Phase 3 trials: DUAL I (IDegLira versus IDeg and Lira 1.8 mg in patients uncontrolled on metformin \pm thiazolidinedione [TZD] for 52 weeks) and DUAL II (IDegLira versus IDeg [max 50 U] in patients uncontrolled on basal insulin + oral glucose lowering drugs for 26 weeks), respectively. This *post-hoc* analysis investigated how the extent of progression of T2D at baseline, defined as baseline HbA_{1c} category, insulin dose and diabetes duration, impacted the efficacy of IDegLira.

Results: In DUAL I, HbA_{1c} reductions (1.1–2.5%) were significantly greater ($p < 0.01$) with IDegLira versus IDeg or Lira in all four baseline HbA_{1c} categories assessed ($\leq 7.5\%$; $> 7.5\%$ to $\leq 8.5\%$; 8.5 to $\leq 9.0\%$ and $> 9.0\%$). HbA_{1c} reductions were similar to those observed in DUAL II (0.9–2.5%) (Figure). In DUAL I, higher baseline HbA_{1c} categories were associated with greater HbA_{1c} reductions with IDegLira ($p < 0.0001$). HbA_{1c} reduction was independent of diabetes duration. In DUAL II, pre-trial basal insulin dose (≤ 30 U or > 30 U) did not affect HbA_{1c} reduction with IDegLira (1.9 vs 1.9%, estimated treatment difference (ETD) 0.02, 95% CI -0.26 to 0.30, $p = 0.91$). Those patients entering DUAL II on metformin (48%) achieved a greater HbA_{1c}

reduction (2.1 vs 1.7%, ETD -0.44, 95% CI -0.71 to -0.16, $p<0.01$) than the remaining 52% who entered on metformin + sulphonylureas (SU) or glinides and then discontinued SU or glinide at randomisation.

Conclusion: HbA_{1c} reductions with IDegLira were substantial for patients with all levels of baseline HbA_{1c} and were independent of diabetes duration and prior insulin dose. Similar levels of glycaemic control can be obtained with IDegLira irrespective of stage of progression of T2D

HbA_{1c} reductions across baseline HbA_{1c} categories in A. DUAL I; B. DUAL II



Clinical Trial Registration Number: NCT01336023, NCT01392573
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IDegLira, a combination of insulin degludec and liraglutide, enables patients with type 2 diabetes to reach target glycaemic control faster than its individual components alone

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Background and aims: IDegLira is a novel combination of insulin degludec (IDeg) and liraglutide (Lira). The aim of this *post hoc* analysis was to investigate the time to glycaemic control and to observe changes in body weight in response to IDegLira vs Lira or IDeg alone, in patients with type 2 diabetes (T2D).

Materials and methods: In two completed phase 3 trials: DUAL I (IDegLira vs IDeg and Lira in patients uncontrolled on oral antidiabetic drugs [OADs]; 52 weeks) and DUAL II (IDegLira vs IDeg in patients uncontrolled on basal insulin + OADs; 26 weeks), a greater HbA_{1c} reduction was observed with IDegLira compared with IDeg or Lira alone (DUAL I: IDegLira: -1.84%, IDeg: -1.40%, Lira: -1.21%; DUAL II: IDegLira: -1.90%, IDeg: -0.89%). In this analysis of both trials, we compared the proportion of patients achieving HbA_{1c} (<7%) and fasting plasma glucose (FPG) (≤7.2 mmol/L) targets and mean change in body weight during the first 12 weeks of treatment. HbA_{1c} was assessed after 8 and 12 weeks; FPG and weight were assessed at Weeks 4, 8 & 12. All data were analysed using LOCF to account for missing data.

Results: At baseline, HbA_{1c} was well matched across treatment arms in both trials. In DUAL I, the proportion of patients achieving HbA_{1c} <7% at Week 8 was significantly greater with IDegLira (57%) than with IDeg (38%) or Lira (47%). These differences in proportions were more pronounced at Week 12. In DUAL II, the proportion of patients at HbA_{1c} target was significantly higher with IDegLira than with IDeg at Weeks 8 & 12. At baseline, the proportion of patients with FPG ≤7.2 mmol/L was similar in both trials. In DUAL I, the proportion of patients at target at Week 4 was significantly higher with IDegLira (76%) than with IDeg (62%) and Lira (62%); these differences persisted at Weeks 8 & 12. In DUAL II, patients were also more likely to achieve FPG target at Weeks 4, 8 & 12. In DUAL I at Weeks 4, 8 & 12, treatment with IDegLira resulted in a significantly greater reduction in mean body weight compared with IDeg, which was associated with a small overall weight gain, but the weight loss with IDegLira was less than that achieved with Lira. In DUAL II, weight loss was greater with IDegLira vs IDeg at Weeks 4, 8 & 12.

Conclusion: Patients treated with IDegLira were more likely to achieve HbA_{1c} and FPG targets earlier than with IDeg or Lira alone. IDegLira provided a favourable early change in weight when compared with IDeg alone, but less than with Lira alone. Treatment with IDegLira results in rapid and substantial improvement in glycaemic control with a beneficial weight profile as early as 4 weeks after initiation in both insulin-naïve and insulin-treated patients. Early improvement in achieving target glycaemia levels may have a positive impact on patients' perception of their progress and on adherence to treatment.

		DUAL I (n=1660)				DUAL II (n=398)			
		IDegLira n=833	IDeg n=413	Lira n=414	IDegLira vs. IDeg, OR* [95% CI]	IDegLira vs. Lira, OR* [95% CI]	IDegLira n=199	IDeg n=199	IDegLira vs. IDeg, OR* [95% CI]
HbA _{1c} <7%, % patients	Week 8	57	38	47	3.06 [2.28; 4.12] $p<0.0001$	1.88 [1.41; 2.50] $p<0.0001$	22	7	4.31 [2.13; 8.72] $p<0.0001$
	Week 12	77	55	60	3.37 [2.51; 4.52] $p<0.0001$	2.90 [2.16; 3.88] $p<0.0001$	45	14	5.99 [3.51; 10.23] $p<0.0001$
FPG ≤7.2 mmol/L, % patients	Week 4	76	62	62	2.22 [1.67; 2.95] $p<0.0001$	2.42 [1.82; 3.23] $p<0.0001$	56	36	3.07 [1.92; 4.90] $p<0.0001$
	Week 8	85	75	61	1.83 [1.34; 2.50] $p<0.0002$	4.34 [3.22; 5.86] $p<0.0001$	70	55	2.41 [1.52; 3.82] $p=0.0179$
	Week 12	87	80	62	1.59 [1.13; 2.22] $p<0.0001$	4.99 [3.65; 6.82] $p<0.0001$	73	62	1.71 [1.10; 2.67] $p=0.0179$
Mean weight change from baseline, kg (SD)	Week 4	-0.6 (1.7)	-0.1 (1.6)	-1.5 (1.8)	-0.43 [-0.63; -0.23] $p<0.0001$	0.95 [0.75; 1.15] $p<0.0001$	-1.4 (1.9)	-0.7 (1.6)	-0.71 [-1.05; -0.37] $p<0.0001$
	Week 8	-0.8 (2.1)	0.1 (2.3)	-2.3 (2.4)	-0.96 [-1.22; -0.69] $p<0.0001$	1.46 [1.19; 1.72] $p<0.0001$	-1.8 (2.5)	-0.6 (2.2)	-1.18 [-1.63; -0.72] $p<0.0001$
	Week 12	-0.9 (2.6)	0.5 (2.8)	-2.6 (2.6)	-1.41 [-1.72; -1.10] $p<0.0001$	1.73 [1.42; 2.04] $p<0.0001$	-2.2 (2.9)	-0.5 (2.6)	-1.65 [-2.18; -1.11] $p<0.0001$

Clinical Trial Registration Number: NCT01336023, NCT01392573
 Supported by: Novo Nordisk

PS 064 GLP-1 based therapies: efficacy II

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Harmony 4: 3 year efficacy of albiglutide (albi) vs insulin glargine (glar) in patients with type 2 diabetes mellitus

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Background and aims: This 3 year (y) randomized, open label, Ph 3 study assessed efficacy and safety of Albi 30 mg once weekly vs Glar in T2DM pts uncontrolled (A1c 53.0–85.8 mmol/mol [7–10%]) on metformin (met) ± sulfonylurea (SU).

Materials and methods: Pts were treated to a target A1c ≤53.0 mmol/mol (7.0%) and FPG ≤5.6 mmol/L (100 mg/dL). If needed, pts could uptitrate Albi to 50 mg QW and Glar per prespecified criteria. Patients could continue if hyperglycemic rescue was required. Primary endpoint (PE) was change from baseline in A1c at wk 52 in Albi vs Glar.

Results: Baseline demographics were similar between groups; mean age 56 y, A1c 67.2 mmol/mol (8.3%), 82% on met + SU. Treated N=504/241 (Albi/Glar). A1c decreased in both groups and was maintained for 3 y. The PE showed non-inferiority between Albi and Glar at wk 52 with a treatment difference of 1.20 mmol/mol (95% CI: 0.44, 2.95) (0.11% [95% CI: -0.04%, 0.27%]). At wk 156, a similar proportion of patients in each group required rescue: Albi 56.2%, Glar 48.0%, p=0.1515. In pts completing 3 y with and without rescue, A1c reduction was durable in both groups. Change in FPG favored Glar, whereas change in weight favored Albi. AEs through wk 156 for Albi/Glar were (% pts): nausea 13.3%/7.5%; diarrhea 10.9%/7.9%; vomiting 5.4%/5.4%. Prerescue documented (≤3.9 mmol/L [70 mg/dL]) symptomatic and severe hypoglycemia events were less with Albi vs Glar (18.5%/0.6% vs 30.3%/0.8%). Injection site reactions occurred in 17.5% of Albi and 10.0% of Glar pts.

Conclusion: Albi showed similar A1c improvement at wk 156 to Glar with modest weight loss and acceptable tolerability.

Table: Change From Baseline Without and With Hyperglycemic Rescue for Key Efficacy Parameters at Week 156

	Without Rescue Albiglutide	Without Rescue Insulin Glargine	With Rescue Albiglutide	With Rescue Insulin Glargine
HbA _{1c} , mmol/mol	n=123	n=88	n=273	n=162
Baseline Mean (SD)	62.51 (8.42)	63.71 (8.75)	66.55 (9.84)	67.32 (10.06)
Week 156 CFB mean (SD)	-9.07 (10.71)	-10.93 (10.06)	-6.01 (14.76)	-8.31 (12.57)
HbA _{1c} , %	n=123	n=88	n=273	n=162
Baseline Mean (SD)	7.87 (0.77)	7.98 (0.80)	8.24 (0.90)	8.31 (0.92)
Week 156 CFB mean (SD)	-0.83 (0.98)	-1.00 (0.92)	-0.55 (1.35)	-0.76 (1.15)
FPG, mmol/L	n=119	n=86	n=264	n=159
Baseline Mean (SD)	8.5 (2.83)	8.8 (2.74)	9.2 (2.81)	9.6 (3.00)
Week 156 CFB mean (SD)	-0.8 (2.80)	-2.2 (3.42)	-1.0 (3.38)	-2.4 (3.66)
FPG, mg/dL	n=119	n=86	n=264	n=159
Baseline Mean (SD)	152.3 (51.00)	158.9 (49.32)	166.3 (50.67)	172.5 (53.93)
Week 156 CFB mean (SD)	-15.0 (50.61)	-39.3 (61.72)	-17.8 (60.90)	-43.3 (65.89)
Weight, kg	n=122	n=89	n=271	n=163
Baseline Mean (SD)	94.19 (18.47)	91.65 (17.40)	94.50 (18.86)	94.27 (18.72)
Week 156 CFB mean (SD)	-3.47 (6.30)	0.90 (4.89)	-1.53 (6.27)	2.01 (5.61)

CFB=change from baseline; FPG=fasting plasma glucose; Ins=insulin; SD=standard deviation.
 n=number of patients with a value who did or did not require rescue.

Clinical Trial Registration Number: NCT00838916

Supported by: GSK

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Harmony 5 - year 3 Results: albiglutide vs placebo and vs pioglitazone in triple therapy (background metformin and glimepiride) in people with type 2 diabetes

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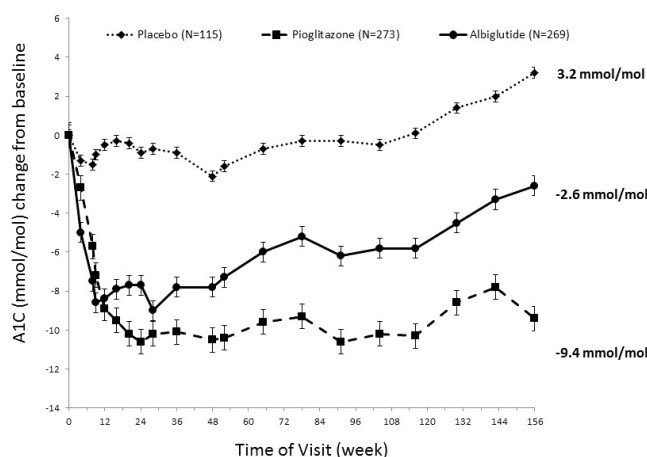
Background and aims: A 3-year, randomized, double-blind, multicenter study evaluated once weekly (QW) GLP-1 receptor agonist albiglutide (Albi) vs placebo (Pbo) and vs pioglitazone (Pio) in people with A1c 53–85.8 mmol/mol [7.0–10.0 %] on background dual therapy of metformin and glimepiride.

Materials and methods: Uptitration of Albi 30 to 50 mg QW and Pio 30 to 45 mg QD was allowed if needed. If hyperglycemic rescue was required, people remained in the study.

Results: Baseline characteristics were similar between groups. At Week 52 (primary endpoint), Albi significantly decreased A1c vs Pbo but did not meet criteria for noninferiority vs Pio. Among people remaining in the study for 3-years (including those who received rescue medication), glycemic control was enhanced on Albi and Pio and maintained on Pbo (figure). Weight loss (kg) was observed on Albi and Pbo (-0.6 and -0.6), but weight increased on Pio (5.9 kg). Adverse events were higher on Albi than Pbo and Pio for injection site reactions (14.4%, 5.2%, 5.1% of participants, respectively), diarrhea (13.7%, 10.4%, 8.3%), and nausea (11.1%, 6.1%, 7.2%), but not vomiting (2.6%, 3.5%, 5.1%). The incidence of SAEs (14.4%, 18.3%, 17.3%) and AEs leading to withdrawal (8.1%, 10.4%, 10.5%) was lower on Albi than Pbo and Pio.

Conclusion: Albi in triple therapy gives effective glucose lowering to 3-years, was well tolerated, and was associated with less weight gain than Pio.

Change from baseline in A1C (mean [SE] mmol/mol) (intent-to-treat population, including postrescue)



Clinical Trial Registration Number: NCT00839527

Supported by: Research funded by GSK

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A network meta-analysis to compare once weekly dulaglutide versus other GLP-1 receptor agonists in patients with type 2 diabetes

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Background and aims: A network meta-analysis (NMA) was performed to compare the efficacy of once weekly dulaglutide 1.5mg to other GLP-1 receptor agonists (GLP1 RAs) licensed for use in the USA/Europe for the treatment of hyperglycaemia in type 2 diabetes (T2D).

Materials and methods: A systematic review was conducted in CENTRAL, EMBASE, MEDLINE and recent conference proceedings from ADA, EASD, IDF-WDC, IDF-WPR and AASD (1990-Jan 2014) to identify randomised controlled trials of adults with T2D treated with liraglutide (1.2mg QD, 1.8mg QD), exenatide (5µg BID, 10µg BID, 2mg QW), lixisenatide (20µg QD) and/or albiglutide (30mg QW, 50mg QW). The AWARD 1, 2, 5 and 6 trials provided data for once weekly dulaglutide 1.5mg. The primary endpoint of the NMA was relative reduction in HbA_{1c}. To adjust for potential heterogeneity, networks were stratified by background therapy: metformin monotherapy (add-on to MET) and metformin in combination with sulfonylurea (SU) and/or thiazolidinediones (TZD) (add-on to MET ± SU ± TZD). Networks were further stratified by study duration of 16–36 weeks and 37–56 weeks to generate four networks. Baseline HbA_{1c} was adjusted for using meta-regression. The NMA was performed within a Bayesian framework; both fixed and random effects models were assessed.

Results: Baseline HbA_{1c} adjusted results are presented as mean differences in HbA_{1c} (%) change from baseline and 95% credible intervals (CrI) for once weekly dulaglutide 1.5mg versus other GLP1 RAs in Table 1. Random effects

models are presented where convergence was achieved; otherwise, fixed effects models are presented. The 37–56 weeks add-on to MET network was disconnected; therefore a NMA could not be conducted. In the 16–36 week network meta-analyses, once weekly dulaglutide 1.5mg showed improved control of HbA_{1c} (%) compared to exenatide 5µg BID and lixisenatide 20µg QD as add-on to both MET and MET ± SU ± TZD, and exenatide 10µg BID as add-on to MET ± SU ± TZD only with statistically significant reductions from baseline. In the 37–56 week add-on to MET ± SU ± TZD NMA, once weekly dulaglutide 1.5mg showed a significant reduction in HbA_{1c} (%) compared to albiglutide 30mg QW and exenatide 10µg BID. A higher baseline HbA_{1c} (%) was associated with greater reductions in HbA_{1c} (%) but the effect was non-significant.

Conclusion: In the AWARD clinical trial program, once weekly dulaglutide 1.5mg demonstrated significant improvements in HbA_{1c} (%) reduction from baseline. In these NMAs, we have shown statistically significant improvements in HbA_{1c} (%) control with once weekly dulaglutide 1.5mg in seven comparisons. The results in the remaining comparisons were not statistically significant. Once weekly dulaglutide 1.5mg was not statistically significantly inferior to other GLP1 RAs in any of the comparisons.

Table 1: Mean difference (95% CrI) in HbA_{1c} (%) change for once weekly dulaglutide 1.5mg vs. comparator

Once weekly dulaglutide 1.5mg vs. comparator*	Analyses		
	Add-on to MET 16–36 weeks (Random Effects)	Add-on to MET ± SU ± TZD 16–36 weeks (Random Effects)	Add-on to MET ± SU ± TZD 37–56 weeks (Fixed Effects)
Albiglutide 30mg QW	-0.22(-0.87, 0.43)	NA	-0.56(-0.78, -0.35)
Albiglutide 50mg QW (titrated)	NA	-0.48(-1.16, 0.21)	NA
Exenatide 10µg BID	-0.44(-0.95, 0.06)	-0.47(-0.81, -0.13)	-0.57(-0.77, -0.37)
Exenatide 5µg BID	-0.76(-1.34, -0.18)	-0.68(-1.29, -0.07)	NA
Exenatide 2mg QW	0.07(-0.38, 0.53)	-0.14(-0.57, 0.31)	NA
Liraglutide 1.2mg QD	-0.25(-0.59, 0.09)	-0.33(-0.97, 0.30)	NA
Liraglutide 1.8mg QD	-0.15(-0.43, 0.13)	-0.27(-0.70, 0.17)	NA
Lixisenatide 20µg QD	-0.62(-1.14, -0.09)	-0.66(-1.12, -0.21)	NA

Abbreviations: QW: once weekly, BID: twice daily, QD: once daily

* Negative difference indicates incremental HbA_{1c} benefit for once weekly dulaglutide 1.5mg; CrI < 0 indicates significant reduction in HbA_{1c} for once weekly dulaglutide 1.5mg

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EVIDENCE 1

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Background and aims: We report 2-year data on effectiveness and tolerability of the GLP-1 analogue liraglutide in the EVIDENCE observational, postmarketing study.

Materials and methods: EVIDENCE is a 2-year multicentre, observational, postmarketing outpatient study requested by the French National Health Authority in order to evaluate the efficacy and safety of liraglutide in clinical practice. Diabetologists and general practitioners in France recruited patients starting treatment with liraglutide. Patients and physicians completed questionnaires at study entry, 3 months and 6 months, then at 6-month intervals for a further 18 months. The primary objective was to determine the percentage of patients still taking liraglutide and at HbA_{1c} target (<7%) after 2 years. We present here data on effectiveness as measured by change in the proportion of patients with HbA_{1c} <7%, HbA_{1c}, fasting plasma glucose and weight. Tolerability and persistence under treatment are also presented. The characteristics of included participants were described by the mean and standard deviation (mean±SD) for continuous measures, and frequency for categorical measures. The changes from baseline to the end of the study were compared by the McNemar test for proportion, and by the Wilcoxon test for continuous measures.

Results: Baseline data were collected from 3152 patients (53% male, age 59±11 years, BMI 34±7 kg/m², diabetes duration 10±6 years, HbA_{1c} 8.5±1.5%); 2029 patients (64.4%) were still taking liraglutide at the end of the study, beyond the initial objective of 1707. Most patients (n=2804, 90%) exceeded the ADA/EASD target with an HbA_{1c} ≥7% at baseline. The proportion of patients with HbA_{1c} <7% was significantly higher after 2 years' liraglutide treatment (n=759, 39.4%) vs. baseline (n=213, 11.0%; p<0.0001).

Following 2 years of liraglutide treatment, significant reductions in HbA_{1c} (-1.01±1.54%, p<0.0001), fasting plasma glucose (-0.32±0.63 g/l, p<0.0001) and body weight (-4.09±6.97 kg, p<0.0001) were observed from baseline. These data are from patients who had both baseline and 2-year data available and were still on liraglutide treatment at the end of the study (n=1928). GI disorders (nausea, vomiting, diarrhoea) were the most frequent adverse events, reported by 261 patients (8.7%) treated with liraglutide; they were also the most common reason for withdrawal.

Conclusion: These results show that the results obtained at 1 year were maintained at the end of the study. The effectiveness of liraglutide in real world clinical practice is similar to efficacy observed in randomised clinical trials (RCTs) (up to -1.5% HbA_{1c} reductions and -3.24 kg weight loss). However, the absence of a control arm makes it difficult to evaluate if observed improvements are attributable to liraglutide alone. The slightly lower mean HbA_{1c} in this study may be due to differing treatment strategies: in RCTs, liraglutide was generally added on to existing therapy, whereas in EVIDENCE a third of patients switched from a DPP-4 inhibitor to liraglutide. The incidence of GI events was lower than that reported in RCTs (up to 26.5%). In summary, 2-year results from the EVIDENCE study suggest that clinical trial data for liraglutide translate into therapeutic benefits in clinical practice.

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Lixisenatide is effective and well tolerated in patients with type 2 diabetes mellitus and renal impairment

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Background and aims: This post hoc assessment evaluated the efficacy and safety of the once-daily prandial glucagon-like peptide-1 (GLP-1) receptor agonist (RA) lixisenatide in patients with type 2 diabetes mellitus and renal impairment.

Materials and methods: Patients from the modified intent-to-treat populations of nine GetGoal trials were categorized by baseline estimated GFR (eGFR) according to ADA 2013 categories of renal function at randomization (normal renal function, eGFR ≥90 ml/min; mild renal impairment [Stage II], eGFR 60–89 ml/min; moderate renal impairment [Stage III], eGFR 30–59 ml/min). Meta-analyses were performed using effect estimates (placebo-adjusted mean difference or placebo-adjusted rate difference for lixisenatide-treated patients between baseline renal categories) and their standard errors, entered as generic inverse variances.

Results: Patient distribution across renal function categories was as follows: normal renal function, lixisenatide n=2081, placebo n=1158; mild renal impairment, lixisenatide n=592, placebo n=404; moderate renal impairment, lixisenatide n=110, placebo n=62. HbA_{1c}, 2h postprandial plasma glucose and fasting plasma glucose were reduced in lixisenatide-treated patients with normal and Stage I, II and III renal function. Meta-analyses showed no significant difference between normal renal function and mild or moderate renal impairment groups for clinical endpoints (Table). The most common adverse events in all groups were gastrointestinal (GI); mainly nausea and vomiting. There was 10% higher risk of nausea and vomiting between patients with normal renal function and those with mild renal impairment (placebo-adjusted rate difference p=0.003), but no significant difference between patients with mild and moderate renal impairment (p=0.92).

Conclusion: This study shows that a difference in baseline renal status did not have an effect on efficacy outcomes in lixisenatide- vs placebo-treated patients, and a uniform placebo-adjusted effect of lixisenatide was observed across renal categories.

Clinical endpoint	N studies	Placebo-adjusted (end of treatment–baseline) difference in changes from baseline between baseline renal categories			
		Normal vs mild impairment		Mild vs moderate impairment	
		Effect estimate (CI)	p-value*	Effect estimate (CI)	p-value*
HbA _{1c} (%)	9	−0.04 (−0.18, 0.10)	0.58	0.15 (−0.17, 0.46)	0.36
2h PPG (mg/dL)	7	4.49 (−19.75, 28.73)	0.72	15.09 (−39.40, 69.59)	0.59
FPG (mg/dL)	9	−5.01 (−11.23, 1.22)	0.12	6.37 (−7.58, 20.32)	0.37

*Random effect p-value

Definitions: Normal renal function, eGFR ≥90 ml/min; Mild impairment (Stage II), eGFR 60–89 ml/min; Moderate impairment (Stage III), eGFR 30–59 ml/min.

Abbreviations: PPG, postprandial plasma glucose; FPG, fasting plasma glucose; SE, standard error

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Predicting treatment success with exenatide twice daily (EBID) at 6 mo: Are baseline postprandial glucose excursions important?

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Background and aims: Information on predicting glycaemic treatment success in patients (pts) before initiation of specific therapies, particularly GLP-1 receptor agonists, is limited. As EBID effectively suppresses postprandial blood glucose (BG) through mechanisms including decreased gastric emptying, we assessed whether a greater postprandial BG defect was predictive of treatment success for EBID and, conversely, whether a pt with a greater fasting BG defect was less likely to achieve treatment success with EBID.

Materials and methods: A pooled dataset (24–30 wks) from 8 EBID studies that collected self-monitored BG data was used. Treatment success at 6 mo was defined as HbA_{1c} <7% or HbA_{1c} decrease ≥1%. Logistic regression models were applied to analyze these binary outcomes, with baseline HbA_{1c}, post-breakfast (postprandial) BG excursion and pre-breakfast (fasting) BG as independent variables. Odds ratios (ORs) were calculated for baseline HbA_{1c}, post-breakfast BG excursion, and pre-breakfast BG.

Results: 1414 EBID pts were included; mean baseline HbA_{1c} was 8.0%. Most pts (93%) received background therapy with ≥1 other agent. At endpoint, mean reduction in HbA_{1c} was 1.0%; 58% of pts achieved HbA_{1c} <7% and 52% achieved HbA_{1c} decrease ≥1% with EBID. Baseline HbA_{1c} was the most significant baseline predictor of treatment success ($P < 0.0001$). The likelihood of attaining treatment success for every 1% increase in baseline HbA_{1c} given the same baseline pre-breakfast and post-breakfast BG depended on the definition of success (Table); pts close to 7% HbA_{1c} at baseline were more likely to attain HbA_{1c} <7% at endpoint, while pts further from 7% HbA_{1c} at baseline were more likely to achieve HbA_{1c} decrease ≥1% at endpoint. For pts with higher post-breakfast (post-prandial) BG treated with EBID, each 1.11 mmol/L increase was significantly associated with a 7.4% increased likelihood of achieving an HbA_{1c} reduction ≥1% given comparable HbA_{1c} and pre-breakfast BG. Baseline post-breakfast BG excursion did not predict achievement of HbA_{1c} <7%. Conversely, pts with an increased pre-breakfast (fasting) BG were significantly less likely to achieve either goal with EBID; ORs for HbA_{1c} <7% or HbA_{1c} reduction ≥1% decreased by 9 and 12%, respectively, for each 1.11 mmol/L increase given comparable baseline HbA_{1c} and post-breakfast glucose.

Conclusion: A greater postprandial BG defect was predictive of a greater likelihood of achieving an HbA_{1c} reduction ≥1% but not HbA_{1c} <7% with EBID and, conversely, a greater fasting BG defect was associated with less likelihood of achieving HbA_{1c} <7% or an HbA_{1c} reduction ≥1% among those treated with EBID. However, neither postprandial nor fasting BG defects

at baseline predicted treatment success with EBID as effectively as baseline HbA_{1c}.

Odd ratios (95% CI) for treatment success at 6 months		
	Exenatide twice daily (EBID) N=1414	
Baseline characteristic	HbA _{1c} <7%	≥1% decrease in HbA _{1c}
HbA _{1c} [every 1% ↑]	0.34 (0.28, 0.41)	3.38 (2.76, 4.14)
Post-breakfast BG excursion [every 1.11 mmol/L ↑]	1.01 (0.95, 1.07)	1.07 (1.01, 1.14)
Pre-breakfast BG [every 1.11 mmol/L ↑]	0.91 (0.83, 0.99)	0.88 (0.81, 0.97)
BG = blood glucose; 1.11 mmol/L = 20 mg/dL		

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Lixisenatide added to basal insulin reduces glycaemic variability in patients with type 2 diabetes mellitus

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Background and aims: This analysis evaluated the impact on glycaemic variability (GV) of lixisenatide (LIXI) vs placebo (PBO) as add-on to basal insulin ± oral antidiabetes drugs (OADs) in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: Patient-level data from GetGoal-L, GetGoal-Duo1, and GetGoal-L-Asia were pooled: standard deviation (SD); mean amplitude of glycaemic excursions (MAGE); mean absolute glucose (MAG); area under the curve-fasting glucose (AUC-F); and high and low blood glucose indices (HBGI/LBGI) were calculated. Changes in GV metrics over 24 weeks of treatment were compared. Relationships between baseline GV, patient characteristics, and clinical outcomes were assessed.

Results: Data from 1,198 patients were analysed (665 LIXI and 533 PBO); 47.9% of patients were men, mean age was 57.2 years, mean BMI 30.4 kg/m², mean T2DM duration 11.7 years, and mean HbA_{1c} 8.1%. GV significantly decreased at Week 24 with LIXI vs PBO, respectively: SD −8.28 vs −3.89 mg/dL ($p = 0.003$); MAG −6.80 vs −1.48 mg/dL ($p < 0.001$); MAGE −16.02 vs −7.02 mg/dL ($p = 0.003$); HBGI −3.65 vs −0.88 ($p < 0.001$); and AUC-F −298.51 vs −15.49 mg/dL*h ($p < 0.001$). LBGI was unchanged 0.04 vs −0.03 ($p = 0.277$). Higher baseline GV correlated with older age, longer T2DM duration, lower BMI, higher baseline HbA_{1c}, greater postprandial plasma glucose (PPG) reduction, and higher symptomatic hypoglycaemia rates (Table).

Conclusion: When added to basal insulin ± OADs, LIXI significantly reduced GV and PPG excursions vs PBO, without increased risk of hypoglycaemia (as shown by LBGI). This may have relevance in patients with advanced T2DM where GV is increased.

	Age	T2DM Duration	Baseline BMI	Baseline HbA _{1c}	PPG Change From Baseline	No. of Symptomatic Hypoglycaemic Events per Year
Baseline SD	0.1163 (< 0.0001)	0.2247 (< 0.0001)	−0.2879 (< 0.0001)	0.2999 (< 0.0001)	−0.2975 (< 0.0001)	0.1022 (0.0006)
Baseline MAG	0.0852 (0.0079)	0.1836 (< 0.0001)	−0.3143 (< 0.0001)	0.2461 (< 0.0001)	−0.3410 (< 0.0001)	0.0716 (0.0255)
Baseline MAGE	0.1011 (0.0007)	0.1878 (< 0.0001)	−0.2757 (< 0.0001)	0.2759 (< 0.0001)	−0.3294 (< 0.0001)	0.0819 (0.0062)
Baseline HBGI	0.0500 (0.0931)	0.1488 (< 0.0001)	−0.1938 (< 0.0001)	0.5655 (< 0.0001)	−0.4441 (< 0.0001)	−0.0056 (0.8509)
Baseline AUC-F	0.1522 (< 0.0001)	0.0999 (0.0051)	−0.1592 (< 0.0001)	0.1857 (< 0.0001)	−0.2429 (< 0.0001)	0.0465 (0.1753)

Table. Relationship of baseline GV with patient characteristics

Data are Pearson correlation coefficients (p-value)

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Sustained glycaemic control with exenatide once weekly versus insulin glargine: associations with baseline factors and early treatment response

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Background and aims: To assist decision making in type 2 diabetes mellitus therapy, this post hoc analysis explored the association between baseline characteristics or early (≤ 12 week) treatment responses with sustained glycaemic control, comparing the glucagon-like peptide-1 receptor agonist exenatide once weekly (QW) with titrated insulin glargine (glargine).

Materials and methods: Three-year data were analysed from the open-label DURATION-3 study, in which patients received exenatide QW (N=233) or glargine (N=223) in addition to metformin alone or with a sulphonylurea. Loss of, or inability to achieve, HbA1c control was defined as HbA1c $>7\%$ at 2 consecutive visits or HbA1c $>9\%$ at 1 visit after 26 weeks of treatment. Baseline characteristics considered were: age, gender, body mass index, weight, background glucose-lowering medication, disease duration, creatinine clearance, HbA1c, fasting serum glucose (FSG), Homeostasis Model Assessment (HOMA) -S (insulin sensitivity) and HOMA-B (β -cell function). Early treatment responses considered were HbA1c, FSG and weight. Cox proportional hazard models were used to analyse the intention to treat (ITT) population and logistic regression was used to analyse the 3-year Completer population (exenatide QW, N=140; glargine, N=147).

Results: Of the ITT patients, 50% on exenatide QW versus 43% on glargine achieved the sustained glycaemic goal; of 3-year Completers, 43 versus 33% achieved the glycaemic goal. The median duration of HbA1c control was 25.0 versus 16.7 months for exenatide QW versus glargine (ITT; Kaplan-Meier; $P=0.03$). The hazard ratio (glargine/exenatide; Cox model) of inability to achieve the glycaemic goal was 1.4 ($P=0.006$). In 3-year Completers (both treatments), stepwise logistic regression showed that only baseline HbA1c and HOMA-B were significantly associated with sustained glycaemic control. Regardless of therapy, every 1% higher baseline HbA1c ($>7\%$) increased the risk of losing glycaemic control by 90% ($P=0.0002$); every 10% higher baseline HOMA-B decreased the risk of losing glycaemic control by 11% ($P=0.0288$). The rate of weight change in weeks 1–8 was the most relevant early treatment response predictor of HbA1c control ($P=0.0640$). The medium slope of weight change was -0.0308 kg/day for exenatide QW (-0.86 kg in 4 weeks) versus 0.0018 kg/day for glargine (0.05 kg in 4 weeks). The odds (glargine/exenatide QW) of losing glycaemic control if the same weight change was seen in both groups was 1.2 (weight loss) and 1.1 (weight gain), indicating that glargine-treated patients were more likely to lose glycaemic control over time regardless of weight gain or loss. For a 1 kg decrease in weight over weeks 1–4 with exenatide QW, the odds of losing glycaemic control decreased by 30%; for a 1 kg increase in weight over weeks 1–4 with glargine, the odds increased by 29%.

Conclusion: Despite continued glargine titration, more patients achieved sustained glycaemic control with exenatide QW. Baseline HbA1c and HOMA-B significantly impacted the odds of sustained control. The analysis of early treatment responses from this study suggest that the rate of weight change following treatment initiation (weeks 1–8) may potentially predict the chance of patients achieving longer term sustained glycaemic control.

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Daily blood glucose variability with exenatide once weekly versus basal insulin in 3 randomised controlled trials

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Background and aims: Glucose lowering therapy aims to improve overall glycaemic control in patients with type 2 diabetes mellitus. However, data on blood glucose variability with different treatments are scarce. Because basal insulin mainly affects fasting plasma glucose and exenatide once weekly (QW) controls both fasting plasma glucose and postprandial plasma glucose, we hypothesized that daily blood glucose variability would be improved more by exenatide QW than by titrated basal insulin in randomised controlled trials.

Materials and methods: After 26 weeks, 7-point self-monitored blood glucose profiles were assessed in 3 randomised controlled trials of exenatide QW: 2 versus insulin glargine (once daily at night) in global and Japanese populations; 1 versus insulin detemir (once daily at night [90%] or twice daily [10%]). All patients had failed ≥ 1 oral drug; Japanese patients discontinued sulphonylureas at randomisation. Outcomes assessed were mean amplitude of glucose excursion (MAGE; average daily mealtime glucose excursion), blood glucose range (average difference between maximum and minimum daily blood glucose) and blood glucose variability (average SD of daily blood glucose).

Results: In all 3 studies, HbA1c reductions were greater with exenatide QW than basal insulin (-1.5 vs -1.3% $P<0.02$; -1.1 vs -0.7% , $P<0.001$; -1.3 vs -0.9% , $P<0.001$, respectively). Baseline characteristics and laboratory results were well balanced across treatments within studies. Exenatide QW significantly reduced different measures of blood glucose variability (including MAGE, range and variability) more than insulin glargine in both available studies (Table), demonstrating greater consistency in blood glucose over the day. A greater decrease in glucose variability was also observed for exenatide QW versus insulin detemir, although comparison between treatments was not statistically significant in this analysis. Basal insulin reduced pre-breakfast (fasting) blood glucose from baseline more than exenatide QW (-2.54 to -3.41 vs -1.88 to -2.77 mmol/L; $P<0.05$) but the relative effect of exenatide QW increased through the day, with a greater reduction in blood glucose post-dinner versus basal insulin (-2.89 to -4.23 vs -1.84 to -2.54 mmol/L; $P<0.05$). The loss of control with basal insulin in the latter half of the day is consistent with its once daily kinetics.

Conclusion: Exenatide QW improved daily blood glucose variability more than insulin glargine, and lowered fasting blood glucose less - but post-dinner blood glucose more - than both basal insulins (glargine and detemir).

Changes from baseline at 26 weeks			
Exenatide QW vs glargine (Global)	Exenatide QW (N=233)	Glargine (N=223)	P value
MAGE*, mmol/L LS mean change (SEM) n	-0.68 (0.11) 187	-0.22 (0.11) 190	$P=0.002$
BG range†, mmol/L LS mean change (SEM) n	-2.18 (0.16) 208	-1.06 (0.16) 206	$P<0.001$
BG variability‡, mmol/L LS mean change (SEM) n	-0.77 (0.06) 208	-0.30 (0.06) 206	$P<0.001$
Exenatide QW vs glargine (Japanese)	Exenatide QW (N=215)	Glargine (N=212)	
MAGE*, mmol/L LS mean change (SEM) n	-1.47 (0.11) 187	-0.07 (0.10) 192	$P<0.001$
BG range†, mmol/L LS mean change (SEM) n	-2.78 (0.15) 197	-0.63 (0.15) 204	$P<0.001$
BG variability‡, mmol/L LS mean change (SEM) n	-1.09 (0.06) 197	-0.20 (0.06) 204	$P<0.001$
Exenatide QW vs detemir (UK)	Exenatide QW (N=111)	Detemir (N=105)	
MAGE*, mmol/L LS mean change (SEM) n	-0.87 (0.17) 75	-0.43 (0.18) 65	$P=0.080$
BG range†, mmol/L LS mean change (SEM) n	-1.47 (0.26) 83	-1.11 (0.27) 77	$P=0.329$
BG variability‡, mmol/L LS mean change (SEM) n	-0.52 (0.09) 83	-0.38 (0.09) 77	$P=0.288$

P-values are for exenatide QW vs basal insulin. *MAGE is average daily mealtime glucose excursion. †BG range is average difference between max and min daily BG. ‡BG variability is average SD of daily BG. LS=least squared. BG=blood glucose. UK=United Kingdom

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Relationship between changes in postprandial glucagon, patient characteristics, and response to lixisenatide as add-on to oral antidiabetics

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Background and aims: Lixisenatide, a once-daily prandial glucagon-like peptide-1 receptor agonist, reduces postprandial (PP) glycaemic excursions and HbA_{1c}. We report an exploratory analysis of the GetGoal-M and S trials in patients with type 2 diabetes mellitus (T2DM) with different changes in PP glucagon levels in response to lixisenatide treatment.

Materials and methods: Patients (n=423) were stratified by their change in 2 hour PP glucagon level between baseline evaluation and Week 24 of treatment with lixisenatide as add-on to oral antidiabetics (OADs) into groups of Greater Change (GC; n=213) or Smaller Change (SC; n=210) in plasma glucagon levels (median change -23.57 ng/L). ANOVA and Chi-squared tests were used for the comparison of continuous and categorical variables, respectively. Baseline and endpoint continuous measurements in each group were compared using paired *t*-tests.

Results: Mean change from baseline in 2 hour PP glucagon levels for the GC vs SC groups was -47.19 vs -0.59 ng/L (*p*<0.0001), respectively. Patients in the GC group had a shorter mean duration of diabetes (7.3 vs 9.0 years; *p*=0.0036) and lesser OAD use (4.5 vs 5.7 years; *p*=0.0092) than those in the SC group. Patients in the GC group had a greater mean reduction in HbA_{1c} (-1.10 vs -0.67%; *p*<0.0001), fasting plasma glucose (FPG; -25.20 vs -9.30 mg/dL [*p*<0.0001]), PP plasma glucose (PPG; -129.40 vs -78.22 mg/dL [*p*<0.0001]), and a greater drop in weight (-2.27 vs -1.17 kg; *p*=0.0002) and body mass index (-0.84 vs -0.44 kg/m²; *p*=0.0002) than those in the SC group. More patients in the GC group also achieved composite endpoints, including HbA_{1c} <7% with no symptomatic hypoglycaemia and no weight gain (40.38 vs 19.52%; *p*<0.0001), than in the SC group.

Conclusion: Greater reductions in PP glucagon associated with lixisenatide as add-on to OADs in patients with T2DM are also associated with greater reductions in HbA_{1c}, FPG, PPG, and greater weight loss, highlighting the importance of glucagon suppression on therapeutic response.

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PS 065 SGLT2 inhibitors: efficacy

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Glycaemic efficacy of canagliflozin by baseline HbA_{1c} and known duration of type 2 diabetes mellitus

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Background and aims: Canagliflozin (CANA) is a sodium glucose co-transporter 2 (SGLT2) inhibitor that has demonstrated glycaemic efficacy across a range of type 2 diabetes mellitus (T2DM) patient populations. This analysis evaluated the effects of CANA compared with placebo (PBO) on changes in HbA_{1c} based on baseline HbA_{1c} and duration of T2DM using pooled data from 4 clinical studies in patients with T2DM.

Materials and methods: Data were pooled from the 4 randomised, double-blind, 26-week, PBO-controlled, Phase 3 studies that enrolled a general population of patients with T2DM (N = 2,313; mean age, 56.0 y; HbA_{1c}, 8.0%; BMI, 32.1 kg/m²; SBP, 128.4 mmHg). Changes in HbA_{1c} with CANA 100 and 300 mg and PBO were evaluated at 26 weeks in subgroups by baseline HbA_{1c} (<8.0%, 8.0%–<9.0%, and ≥9.0%) and T2DM duration (<5 y, 5–<10 y, and ≥10 y).

Results: CANA 100 and 300 mg, respectively, were associated with progressively greater PBO-subtracted reductions in HbA_{1c} as baseline HbA_{1c} increased (<8.0%: -0.45% and -0.65%; 8.0%–<9.0%: -0.91% and -1.07%; ≥9.0%: -1.25% and -1.48%, Table). CANA 100 and 300 mg were associated with PBO-subtracted reductions in HbA_{1c} in patients with T2DM <5 y duration (-0.70% and -0.96%, respectively) as well as those with 5–<10 y T2DM duration (-0.74% and -0.91%, respectively) and ≥10 y T2DM duration (-0.74% and -0.85%, respectively). Overall AE rates with CANA 100 and 300 mg and PBO were 60.1%, 59.2%, and 59.4%, respectively. CANA 100 and 300 mg were associated with a higher incidence of genital mycotic infections and osmotic diuresis-related AEs (eg, pollakiuria [increased urine frequency], thirst) compared with PBO; these AEs led to few discontinuations.

Conclusion: CANA provided glycaemic improvements in patients with T2DM regardless of baseline HbA_{1c} or known duration of T2DM. Greater reductions in HbA_{1c} with CANA were seen in patients with higher baseline HbA_{1c}, similar to what has been observed with other antihyperglycaemic agents. Reductions in HbA_{1c} were similar across all T2DM duration subgroups.

Table. Change in HbA_{1c} by Baseline HbA_{1c} and T2DM Duration (LOCF)

	HbA _{1c} <8.0%			HbA _{1c} 8.0%–<9.0%			HbA _{1c} ≥9.0%		
	PBO (n = 355)	CANA 100 mg (n = 425)	CANA 300 mg (n = 438)	PBO (n = 179)	CANA 100 mg (n = 272)	CANA 300 mg (n = 281)	PBO (n = 112)	CANA 100 mg (n = 136)	CANA 300 mg (n = 135)
Mean (SD) HbA _{1c} , baseline, %	7.3 (0.4)	7.3 (0.4)	7.3 (0.4)	8.4 (0.3)	8.4 (0.3)	8.4 (0.3)	9.6 (0.5)	9.6 (0.4)	9.6 (0.5)
LS mean (SE) change, %	-0.02 (0.04)	-0.47 (0.03)	-0.66 (0.03)	-0.20 (0.06)	-1.11 (0.05)	-1.28 (0.06)	-0.31 (0.11)	-1.57 (0.10)	-1.80 (0.10)
Difference vs PBO ^a		-0.45 (-0.55, -0.35)	-0.65 (-0.74, -0.55)		-0.91 (-1.07, -0.75)	-1.07 (-1.24, -0.91)		-1.25 (-1.54, -0.97)	-1.48 (-1.77, -1.20)
	T2DM Duration <5 y			T2DM Duration 5–<10 y			T2DM Duration ≥10 y		
	PBO (n = 253)	CANA 100 mg (n = 341)	CANA 300 mg (n = 342)	PBO (n = 200)	CANA 100 mg (n = 264)	CANA 300 mg (n = 245)	PBO (n = 193)	CANA 100 mg (n = 228)	CANA 300 mg (n = 247)
Mean (SD) HbA _{1c} , baseline, %	7.9 (1.0)	7.9 (0.9)	7.9 (0.9)	8.1 (0.9)	8.1 (0.9)	8.0 (0.9)	8.1 (0.9)	8.1 (0.9)	8.2 (1.0)
LS mean (SE) change, %	-0.12 (0.06)	-0.82 (0.05)	-1.08 (0.05)	-0.12 (0.06)	-0.88 (0.05)	-1.03 (0.06)	-0.08 (0.06)	-0.81 (0.06)	-0.92 (0.05)
Difference vs PBO ^a		-0.70 (-0.84, -0.56)	-0.96 (-1.10, -0.82)		-0.74 (-0.90, -0.59)	-0.91 (-1.06, -0.75)		-0.74 (-0.88, -0.58)	-0.85 (-1.00, -0.70)

LOCF, last observation carried forward; SD, standard deviation; SE, standard error; LS, least squares. PBO-subtracted LS mean changes (95% CI).

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Durability of glycaemic response with dapagliflozin as add-on therapy in type 2 diabetes inadequately controlled with metformin; 4-year data versus glipizideS. Del Prato¹, M. Nauck², K. Rohwedder³, E. Johnsson⁴, S. Parikh⁵;¹University of Pisa, Italy, ²Diabetes Center, Bad Lauterberg,³AstraZeneca, Wedel, Germany, ⁴AstraZeneca, Mölndal, Sweden,⁵AstraZeneca, Wilmington, USA.

Background and aims: Dapagliflozin (DAPA), a selective inhibitor of the sodium-glucose co-transporter 2, reduces plasma glucose by increasing renal glucose excretion. Its insulin-independent mechanism results in a low risk of hypoglycaemia in contrast to sulphonylureas. In a phase 3 trial of DAPA (≤ 10 mg/d) versus glipizide (≤ 20 mg/d) in patients (N=814) with type 2 diabetes mellitus inadequately controlled by metformin (MET) alone (median 2000 mg/d), the change in HbA_{1c} with DAPA was statistically noninferior to glipizide over 52 weeks. Further to these previously presented data on the primary end point we report the durability of glycaemic control over 4 years.

Materials and methods: The durability of glycaemic control was evaluated by calculating the coefficient of failure (CoF; the slope of the regression line vs time) for HbA_{1c} and fasting plasma glucose (FPG) from 18 weeks (end of titration period) to 208 weeks. DAPA and glipizide were down-titrated if medically indicated. The CoF was determined for 3 groups: patients who had at least 3 postbaseline values from after week 18 to week 208 (full analysis set), all patients who completed 208 weeks of treatment (all completers), and patients who completed 208 weeks of treatment but who were not down-titrated (completers not down-titrated). Patients who required rescue medication were excluded from the analyses.

Results: The CoF for DAPA was significantly lower compared with glipizide, irrespective of which glycaemic variable (HbA_{1c} or FPG) or analysis set was employed (Table). For the full analysis set, the CoF for HbA_{1c} showed a rise of 0.2%/year (95% CI, 0.1 to 0.3) with DAPA and a rise of 0.6%/year (95% CI, 0.5 to 0.7) with glipizide. Corresponding values for FPG were 0.4 mmol/L/year (95% CI, 0.2 to 0.5) for DAPA and 0.8 mmol/L/year (95% CI, 0.5 to 1.1) for glipizide. More patients treated with DAPA completed the 208-week study than patients treated with glipizide (161 vs 141, respectively). Fewer DAPA-treated patients had their dose down-titrated (3 vs 42, respectively; Table). For all completers and for completers who were not down-titrated, the CoFs for HbA_{1c} and FPG were significantly lower with DAPA compared with glipizide (Table). Thus, the higher rates of discontinuation or down-titration with glipizide did not account for the lower CoF with DAPA. Treatment with DAPA in combination with MET was well tolerated over the 208-week period.

Conclusion: Glycaemic durability over a 4-year period was significantly better with DAPA compared with glipizide as demonstrated by the CoF. DAPA in combination with MET was well tolerated.

Analysis set [n: DAPA vs glipizide]	Difference: DAPA versus glipizide	
	HbA _{1c} , %/year (95% CI)	FPG, mmol/L/year (95% CI)
Full analysis set [n: 334* vs 332]	-0.4 (0.6 to -0.3) P=0.0001	-0.4 (-0.8 to -0.1) P=0.0069
All completers [n: 204 vs 188]	-0.2 (-0.2 to -0.1) P=0.0001	-0.2 (-0.3 to -0.1) P=0.0037
Completers not down-titrated [n: 201 vs 146]	-0.2 (-0.3 to -0.1) P<0.0001	-0.2 (-0.4 to -0.1) P=0.0019

*n for DAPA FPG = 333.

Table: Difference in HbA_{1c} and FPG CoF for DAPA versus glipizide from week 18 to week 208.

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Empagliflozin twice daily versus once daily as add-on to metformin in patients with type 2 diabetesS. Ross¹, C. Thamer², J. Cescutti³, T. Meinicke⁴, H.J. Woerle⁴, U.C. Broedl⁴;¹University of Calgary, LMC Endocrinology Centres, Canada,²Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany,³Boehringer Ingelheim France S.A.S., Reims, France,⁴Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany.

Background and aims: A randomized, double-blind, placebo-controlled, parallel-group study compared the efficacy and safety of empagliflozin (EMPA) twice daily (bid) versus once daily (qd) regimens as add-on to metformin (MET) in patients with type 2 diabetes (T2DM).

Materials and methods: Patients (n=983) were randomised to EMPA 12.5 mg bid (n=219), EMPA 25 mg qd (n=218), EMPA 5 mg bid (n=219), EMPA 10 mg qd (n=220) or placebo (PBO; n=107) added on to stable dose MET bid (≥ 1500 mg/day) for 16 weeks. The primary endpoint was change from baseline in HbA_{1c} at week 16. The secondary endpoint was change from baseline in fasting plasma glucose (FPG) at week 16. Exploratory endpoints included changes from baseline in body weight, systolic and diastolic blood pressure (SBP and DBP) at week 16. The primary analysis was a test of non-inferiority of EMPA 12.5 mg bid versus EMPA 25 mg qd and of EMPA 5 mg bid versus EMPA 10 mg qd. The superiority of EMPA doses versus placebo was also tested. Efficacy was evaluated in 965 patients (mean [SD] age 58.2 [10.3] years; weight 89.0 [18.5] kg; BMI 31.8 [5.2] kg/m²; HbA_{1c} 7.77 [0.80] %).

Results: Reductions from baseline in HbA_{1c} were non-inferior for EMPA 12.5 mg bid versus 25 mg qd and for EMPA 5 mg bid versus 10 mg qd. Reductions from baseline in HbA_{1c} were statistically significant with all EMPA doses compared with PBO. FPG changes with EMPA were consistent with HbA_{1c} changes. EMPA qd and bid doses reduced body weight, SBP and DBP to a similar extent. Adverse events (AEs) were reported by 45.7%, 41.7%, 43.8%, 50.0% and 47.7% of patients on EMPA 12.5 mg bid, EMPA 25 mg qd, EMPA 5 mg bid, EMPA 10 mg qd and PBO, respectively. Confirmed hypoglycaemic AEs (<70 mg/dl and/or requiring assistance) were reported by 1 patient each on EMPA 25 mg qd, EMPA 5 mg bid, EMPA 10 mg qd and PBO, and were generally mild in intensity; none required assistance. AEs consistent with urinary tract infection (UTI) were reported in 5.9%, 5.5%, 7.8%, 9.5% and 3.7% of patients on EMPA 12.5 mg bid, EMPA 25 mg qd, EMPA 5 mg bid, EMPA 10 mg qd and PBO, respectively. AEs consistent with genital infection were reported in 4.1%, 4.1%, 3.7%, 3.2% and 2.8% of patients in these groups, and increased urination in 2.7%, 2.8%, 2.7%, 2.3% and 1.9% of patients in these groups, respectively. AEs consistent with volume depletion were reported in 0.5% of patients on EMPA 12.5 mg bid, 0.9% on EMPA 10 mg qd and no patients in other groups.

Conclusion: As add-on to MET in patients with T2DM, HbA_{1c} reductions with EMPA bid dose regimens were statistically non-inferior to those with EMPA qd dose regimens. All EMPA dose regimens led to significant reductions in HbA_{1c} versus PBO and were well tolerated.

	EMPA 12.5 mg bid (N=215)	EMPA 25 mg qd (N=214)	EMPA 5 mg bid (N=215)	EMPA 10 mg qd (N=214)	PBO (N=107)
Baseline HbA _{1c} (%)	7.78 (0.05)	7.73 (0.05)	7.79 (0.06)	7.83 (0.05)	7.69 (0.07)
Change from baseline in HbA _{1c} at week 16 (%)	-0.83 (0.05)	-0.72 (0.05)	-0.66 (0.05)	-0.64 (0.05)	-0.22 (0.07)
Difference vs. EMPA qd regimen (95% CI)	-0.11 (-0.26, 0.03)***	–	-0.02 (-0.16, 0.13)***	–	–
Difference vs. PBO (95% CI)	-0.61 (-0.79, -0.44)†	-0.50 (-0.68, -0.32)†	-0.44 (-0.62, -0.27)†	-0.42 (-0.60, -0.25)†	–
Baseline FPG (mg/dl)	156.7 (2.6)	157.6 (2.2)	162.7 (2.8)	160.7 (2.7)	159.8 (3.3)
Change from baseline in FPG at week 16 (mg/dl)	-27.7 (2.0)	-22.7 (2.0)	-21.2 (2.0)	-17.6 (2.0)	-0.2 (2.8)
Baseline body weight (kg)	89.4 (1.3)	88.7 (1.3)	88.3 (1.2)	89.1 (1.3)	90.1 (1.8)
Change from baseline in body weight at week 16 (kg)	-3.7 (0.2)	-2.9 (0.2)	-2.9 (0.2)	-2.7 (0.2)	-1.0 (0.3)
Baseline SBP (mmHg)	130.2 (1.0)	131.0 (1.0)	132.4 (1.0)	131.6 (1.0)	131.5 (1.4)
Change from baseline in SBP at week 16 (mmHg)	-4.1 (0.7)	-3.8 (0.7)	-4.2 (0.7)	-2.5 (0.8)	1.6 (1.1)

Baseline values are mean (SE). Changes are adjusted mean (SE) based on an analysis of covariance (ANCOVA) performed in patients treated with ≥ 1 dose of trial medication who had a baseline and an on-treatment HbA_{1c} value with last observation carried forward imputation (LOCF). Results of statistical testing only shown for change from baseline in HbA_{1c} (the primary endpoint). ***p<0.001 for non-inferiority, tested using the Hochberg procedure to control type I error at 0.025 (one-sided), with margin of 0.35%; †p<0.001 for superiority.

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Systematic review and network meta-analysis to compare dapagliflozin with other diabetes medications in combination with metformin for adults with type 2 diabetes

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Background and aims: Effective management of type 2 diabetes mellitus (T2DM) and its complications is required in order to address the growing burden of the disease on healthcare resources. However, there are many factors to consider when assessing the suitability of available treatments. A network meta-analysis (NMA) update was undertaken to evaluate the sodium glucose co-transporter-2 (SGLT-2) inhibitor, dapagliflozin, versus other diabetes medications as add-on to metformin. This update allowed inclusion of a new drug class (glucagon-like peptide-1 [GLP-1] analogues), a new time-point (24-weeks) and covariate analysis.

Materials and methods: The systematic review identified recent randomised controlled trials (2011–July 2013) involving T2DM patients inadequately controlled on metformin monotherapy. Comparators licensed in the EU included dipeptidyl peptidase-4 inhibitors (DPP-4i), thiazolidinediones (TZDs), glucagon-like peptide-1 (GLP-1s) analogues, sulfonylureas (SUs) and dapagliflozin. Bayesian NMA was conducted for outcomes at 24- and 52-weeks, and both fixed and random-effect models were explored. Covariate analyses were performed to assess confounding baseline parameters where feasible.

Results: Of 2247 articles retrieved, 16 were included in the review. Combined with 19 from the pre-2011 analysis, a total of 19 and 8 studies were included in the 24-week and 52-week basecase NMA, respectively. There were sufficient data to analyse mean change in HbA1c, systolic blood pressure (SBP), change in weight, as well as the proportion of patients experiencing hypoglycaemia at both 24 and 52-weeks. There were no significant differences in change in HbA1c or SBP between dapagliflozin and the other classes of diabetes drugs as add-on to metformin at 24 or 52-weeks. Significant weight loss results were seen by 24-weeks for dapagliflozin compared to DPP-4i and TZD and at 52-weeks for dapagliflozin compared to SU, DPP-4i and TZD: -4.66 kg (-6.43, -2.90); -2.59 kg (-4.53, -0.66) and -4.76 kg (-7.28, -2.24) at 52 weeks, respectively. Dapagliflozin resulted in a significantly lower risk of hypoglycaemia compared to SU (OR: 0.05 [0.01, 0.19]) over 52-weeks. Covariate analysis indicated that a higher HbA1c at baseline was predictive of a larger treatment effect.

Conclusion: This NMA update supports previous findings that effects on HbA1c are largely similar between drug classes. However, dapagliflozin compared with DPP-4i, TZDs and SUs offered superior weight control when added to metformin and was associated with a significantly reduced risk of hypoglycaemia in comparison to SUs. The wider evidence base compared to previous analysis increases the confidence in the results.

Table 1 Summary of results from basecase NMA: Dapagliflozin head-to-head with other drug classes

Outcome	Difference in HbA1c	Difference in weight kg	Difference in SBP	OR of hypoglycaemia
24 week basecase	RE NMA with 3 covariates*	RE NMA	RE NMA	RE NMA
Dapagliflozin v GLP1	0.30 (-0.21, 0.81)	-0.61 (-1.69, 0.46)	-2.16 (-6.73, 2.19)	0.79 (0.16, 3.82)
Dapagliflozin v DPP4	0.07 (-0.42, 0.55)	-2.24 (-3.25, -1.24)*	-3.22 (-7.65, 0.93)	1.08 (0.24, 4.74)
Dapagliflozin v TZD	0.25 (-0.30, 0.81)	-4.65 (-5.89, -3.45)*	-2.69 (-8.52, 2.72)	2.56 (0.26, 33.50)
Dapagliflozin v placebo	-0.60 (-1.06, -0.16)*	-2.04 (-2.97, -1.12)*	-3.76 (-7.15, -0.41)*	0.99 (0.25, 3.89)
Dapagliflozin v baseline	-0.68 (-1.15, -0.22)*	-2.89 (-3.86, -1.93)*	-5.23 (-9.58, -0.89)*	-
52 week basecase	RE NMA (no covariates)	RE NMA	FE Bucher IC	RE NMA
Dapagliflozin v GLP1	0.41 (-0.01, 0.84)	-0.53 (-3.05, 2.00)	-1.48 (CI: -6.01, 3.05)	-
Dapagliflozin v DPP4	-0.11 (-0.42, 0.22)	-2.59 (-4.53, -0.66)*	-2.20 (CI: -5.97, 1.57)	0.57 (0.14, 2.56)
Dapagliflozin v TZD	-0.02 (-0.45, 0.40)	-4.76 (-7.28, -2.24)*	-	0.57 (0.08, 4.81)
Dapagliflozin v SU	0.00 (-0.29, 0.29)	-4.66 (-6.43, -2.90)*	-5.10 (CI: -8.27, -1.93)†	0.05 (0.01, 0.19)*
Dapagliflozin v baseline	-0.67 (-0.98, -0.36)*	-3.30 (-5.07, -1.53)*	-	-

(95% credible interval); * statistically significant result based on 95% credible interval; † statistically significant p<0.05; + baseline HbA1c, weight and age; CI, 95% confidence interval; DPP4, dipeptidyl peptidase-4; FE, fixed-effect; GLP1, glucagon-like peptide-1; IC, indirect comparison; NA, not applicable; NMA, network meta-analysis; OR, odds ratio; RE, random-effects; SBP, systolic blood pressure; SGLT2, sodium glucose transporter-2; SU, sulfonylureas; TZD, thiazolidinedione

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Fixed dose combinations of empagliflozin and linagliptin for 52 weeks in drug-naïve subjects with type 2 diabetes

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Background and aims: A randomized, double-blind, parallel group Phase III study evaluated the efficacy and safety of fixed dose combinations (FDCs) of empagliflozin/linagliptin (EMPA/LINA) in drug-naïve subjects with type 2 diabetes (T2DM).

Materials and methods: Subjects were randomized to EMPA 25 mg/LINA 5 mg (n=137), EMPA 10 mg/LINA 5 mg (n=136), EMPA 25 mg (n=135), EMPA 10 mg (n=134), or LINA 5 mg (n=135) for 52 weeks. Primary analysis was at week 24. Exploratory endpoints at week 52 were changes from baseline in HbA1c, body weight, systolic and diastolic blood pressure (SBP and DBP), and percentage of subjects with baseline HbA1c ≥7% who reached HbA1c <7%. Efficacy was evaluated in 667 subjects (mean [SD] age 54.6 [10.2] years; weight 87.9 [20.1] kg; BMI 31.6 [5.6] kg/m²; HbA1c 8.02 [0.96] %).

Results: Compared with LINA 5 mg, both FDCs led to significant reductions in HbA1c and higher percentages of subjects with HbA1c <7% at week 52. EMPA 10 mg/LINA 5 mg led to significant reductions in HbA1c and a higher percentage of subjects with HbA1c <7% vs EMPA 10 mg. There were no significant differences in changes from baseline in HbA1c or percentages of subjects with HbA1c <7% with EMPA 25 mg/LINA 5 mg vs EMPA 25 mg. Compared with LINA 5 mg, body weight was significantly reduced with EMPA 25 mg/LINA 5 mg (difference: -2.2 kg [95% CI 3.4, 1.0]; p<0.001) and EMPA 10 mg/LINA 5 mg (difference: 1.5 kg [95% CI -2.8, -0.3]; p<0.05). The FDCs did not reduce weight vs their respective EMPA monotherapies. There were no changes from baseline in SBP or DBP with LINA 5 mg but EMPA and FDCs reduced SBP from baseline by 2.1 to 2.5 mmHg and DBP by 0.2 to 1.6 mmHg. Adverse events (AEs) were reported in 75.7%, 72.8%, 68.9%, 81.5% and 71.9% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively, over 52 weeks. Confirmed hypoglycemic AEs (glucose ≤70 mg/dL and/or requiring assistance) were reported in 1 patient each on EMPA 25 mg and LINA 5 mg and 4 subjects on EMPA 10 mg; none required assistance. AEs consistent with urinary tract infection were reported in 12.5%, 15.4%, 10.4%, 16.3% and 10.4% of subjects on EMPA 25 mg/LINA 5 mg, EMPA 10 mg/LINA 5 mg, EMPA 25 mg, EMPA 10 mg and LINA 5 mg, respectively, and AEs consistent with genital infection were reported in 5.9%, 2.9%, 4.4%, 5.2% and 3.0% of subjects in these groups, respectively.

Conclusion: In drug-naïve subjects with T2DM, FDCs of EMPA 25 mg/LINA 5 mg and EMPA 10 mg/LINA 5 mg for 52 weeks significantly reduced HbA1c vs LINA 5 mg. HbA1c reductions were significantly greater with EMPA 10 mg/LINA 5 mg vs EMPA 10 mg, but not with EMPA 25 mg/LINA 5 mg vs EMPA 25 mg. FDCs were well tolerated, with overall safety profiles similar to those known for the individual components.

	EMPA 25 mg/ LINA 5 mg (n=134)	EMPA 10 mg/ LINA 5 mg (n=134)	EMPA 25 mg (n=133)	EMPA 10 mg (n=131)	LINA 5 mg (n=133)
Baseline HbA1c (%)	7.99 (0.08)	8.02 (0.08)	7.99 (0.08)	8.05 (0.09)	8.05 (0.08)
Change from baseline in HbA1c at week 52 (%)	-1.18 (0.09)	-1.25 (0.09)	-1.02 (0.09)	-0.87 (0.09)	-0.51 (0.09)
Difference vs EMPA 25 mg (95% CI)	-0.16 (-0.41, 0.08)	—	—	—	—
Difference vs EMPA 10 mg (95% CI)	—	-0.38 (-0.63, -0.13)**	—	—	—
Difference vs LINA 5 mg (95% CI)	-0.67 (-0.93, -0.42)***	-0.74 (-0.99, -0.49)***	—	—	—
Subjects with HbA1c >7% at baseline* who had HbA1c <7% at week 24, n (%)	61 (50.4)	62 (50.8)	54 (45.8)	40 (33.1)	35 (27.6)
Odds ratio vs EMPA 25 mg (95% CI)	1.22 (0.72, 2.08)	—	—	—	—
Odds ratio vs EMPA 10 mg (95% CI)	—	2.24 (1.30, 3.85)**	—	—	—
Odds ratio vs LINA 5 mg (95% CI)	2.97 (1.72, 5.15)***	3.08 (1.78, 5.34)***	—	—	—

Baseline values are mean (SE). Changes are adjusted mean (SE) based on mixed model repeated measures approach using observed cases in subjects treated with ≥ 1 dose of trial medication who had a baseline and on-treatment HbA1c value. n=121 for EMPA 25 mg/LINA 5 mg, n=122 for EMPA 10 mg/LINA 5 mg, n=118 for EMPA 25 mg, n=121 for EMPA 10 mg, n=127 for LINA 5 mg. **p<0.01; ***p<0.001.

Clinical Trial Registration Number: NCT01422876

Supported by: Boehringer Ingelheim and Eli Lilly

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Improvement in glycaemic control and reduction in body weight over 52 weeks with dapagliflozin as add-on therapy to metformin plus sulphonylurea

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Background and aims: Dapagliflozin (DAPA), a highly selective sodium-glucose co-transporter 2 inhibitor, improves glycaemic control and promotes body weight loss in patients with type 2 diabetes mellitus (T2DM) as monotherapy or in combination with other antihyperglycaemic drugs. This phase 3 study evaluated DAPA as an add-on therapy to metformin (MET) plus sulphonylurea (SU).

Materials and methods: Patients (men and women aged ≥ 18 years) with T2DM (HbA1c $\geq 7.0\%$ – $\leq 10.5\%$ at randomisation) who were receiving a stable (≥ 8 weeks prior to enrolment) dose combination of MET (≥ 1500 mg/d) and SU (maximum tolerated dose of at least half maximum dose) were eligible to participate. Patients were randomised to receive DAPA 10 mg or placebo (PBO) once daily for 52 weeks (24-week double-blind period and 28-week extension).

Results: At week 52, HbA1c and fasting plasma glucose (FPG) improved with DAPA versus PBO (HbA1c -0.8% vs -0.1%; FPG -1.5 mmol/L [-27.6 mg/dL] vs 0.6 mmol/L [11.5 mg/dL]; Table). Over 52 weeks more patients achieved the American Diabetes Association recommended glycaemic goal of HbA1c < 7.0% with DAPA (27.3%) versus PBO (11.3%). Both body weight and systolic blood pressure were reduced with DAPA versus PBO. Total, low-density lipoprotein, and high-density lipoprotein cholesterol increased and triglycerides decreased from baseline to week 52 with DAPA versus PBO. No patient discontinued as a result of a lack of glycaemic control and fewer patients on DAPA (10.1%) than on PBO (42.7%) were rescued for failing to reach glycaemic targets (week 4–16, FPG >13.2 mmol/L (240 mg/dL); week 16–24, FPG >11.1 mmol/L (200 mg/dL); week 24–52, HbA1c > 8.0%). In DAPA versus PBO groups the frequency of adverse events (AEs) was 69.7% versus 73.4%, respectively; serious AEs were 6.4% versus 7.3%, and hypoglycaemic events were 15.6% versus 8.3% (1 event of hypoglycaemia led to discontinuation in the DAPA treatment arm). Genital infections were reported by 10.1% versus 0.9% of patients with DAPA versus PBO (women 14.3% vs 2.0%; men 4.3% vs 0%). Urinary tract infections were reported by 10.1% versus 11.0% of patients with DAPA versus PBO (women 12.7% vs 22.4%; men 6.5% vs 1.7%).

Conclusion: DAPA 10 mg resulted in sustained glycaemic benefits and weight loss over the duration of this 52-week study in patients with T2DM and inadequate glycaemic control on a background combination of MET and SU.

Longitudinal analysis, excluding data after rescue	PBO (n=108)	DAPA (n=108)
Mean BL HbA1c, % (SD)	8.2 (0.9)	8.1 (0.9)
Adj. mean change from BL to week 52, % (95% CI)	-0.1 (-0.3 to 0.1)	-0.8 (-1.0 to -0.6)
Mean BL FPG, mmol/L [mg/dL] (SD)	10.0 (2.4)	9.3 (2.4)
	[180.5 (43.3)]	[167.4 (43.3)]
Adj. mean change from BL to week 52, mmol/L (mg/dL) (95% CI)	0.6 (0.1 to 1.1)	-1.5 (-1.9 to -1.1)
	[11.5 (2.4 to 20.6)]	[-27.6 (-34.9 to -20.3)]
Mean BL body weight, kg (SD)	90.1 (16.2)	88.6 (17.6)
Adj. mean change from BL to week 52, kg (95% CI)	-1.0 (-1.8 to -0.1)	-2.9 (-3.6 to -2.2)
Mean BL systolic BP mm Hg (SD)	136.3 (14.3)	134.5 (12.6)
Adj. mean change from BL to week 52, mm Hg (95% CI)	1.1 (-2.2 to 4.5)	-1.0 (-3.6 to 1.6)
Mean BL total cholesterol, mmol/L [mg/dL] (SD)	4.5 (0.9)	4.6 (1.2)
	[172.1 (36.0)]	[178.4 (44.8)]
Adj. percent change from BL to week 52, % (95% CI)	1.4 (-2.9 to 6.0)	3.4 (-0.1 to 7.0)
Mean BL LDL, mmol/L [mg/dL] (SD)	2.4 (0.8)	2.5 (1.0)
	[92.7 (30.0)]	[95.8 (38.2)]
Adj. percent change from BL to week 52, % (95% CI)	0.9 (-6.7 to 9.1)	4.8 (-1.5 to 11.5)
Mean BL HDL, mmol/L [mg/dL] (SD)	1.2 (0.3)	1.2 (0.3)
	[45.3 (10.1)]	[47.5 (12.4)]
Adj. percent change from BL to week 52, % (95% CI)	0.6 (-3.6 to 4.9)	6.9 (3.3 to 10.6)
Mean BL triglycerides, mmol/L [mg/dL] (SD)	2.0 (0.9)	2.1 (1.4)
	[174.0 (78.8)]	[184.7 (127.3)]
Adj. percent change from BL to week 52, % (95% CI)	2.9 (-8.1 to 15.2)	-8.0 (-16.0 to 0.7)

Adj., adjusted; BL, baseline; CI, confidence interval; BP, blood pressure; SD, standard deviation; LDL, low density lipoprotein; HDL, high density lipoprotein.

Clinical Trial Registration Number: NCT01392677

Supported by: AZ/BMS

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Efficacy and safety of canagliflozin in patients with type 2 diabetes mellitus who were, or were not, on antihyperglycaemic agents at screening

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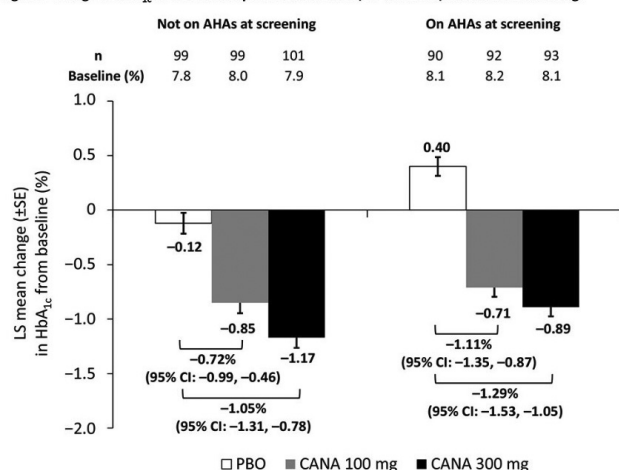
Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, demonstrated glycaemic improvement and was generally well tolerated in a Phase 3 study of patients with type 2 diabetes mellitus (T2DM) inadequately controlled on diet and exercise. In that study, the efficacy and safety of CANA monotherapy compared with placebo (PBO) were evaluated in patients who were, or were not, on antihyperglycaemic agents (AHAs) at screening.

Materials and methods: In this randomised, double-blind, PBO-controlled study, patients with T2DM inadequately controlled with diet and exercise (N = 584; age, 55.4 y; HbA1c, 8.0%; fasting plasma glucose [FPG], 9.5 mmol/L; body mass index, 31.6 kg/m²) received CANA 100 or 300 mg or PBO once daily. Change in HbA1c from baseline to Week 26 was assessed in the overall modified intent-to-treat population and subsets of patients who were not on AHAs at screening (n = 303), or on AHAs (oral AHA monotherapy [except PPAR- γ agonists] or low-dose combination metformin + sulphonylurea) and entered the AHA washout period (n = 281).

Results: Patients on AHAs at screening had a higher baseline mean age and T2DM duration, lower mean estimated glomerular filtration rate (eGFR), and higher HbA1c and FPG. Relative to PBO, CANA 100 and 300 mg lowered HbA1c in patients not on AHAs (differences of -0.72% and -1.05%) and on AHAs at screening (differences of -1.11% and -1.29%; Figure) at Week 26; changes were similar to those in the overall population. The overall incidence of adverse events (AEs) was higher with CANA 100 and 300 mg than PBO in patients not on AHAs (59% and 52% vs 46%) and on AHAs at screening (62% and 68% vs 52%), but the incidence of serious AEs and AEs leading to discontinuation were low across groups in both subsets.

Conclusion: CANA treatment lowered HbA_{1c} and was generally well tolerated in patients with T2DM who were, or were not, on prior AHA therapy at screening.

Figure. Change in HbA_{1c} at Week 26 in patients who were, or were not, on AHAs at screening.



AHA, antihyperglycaemic agent; LS, least squares; SE, standard error; CI, confidence interval; PBO, placebo; CANA, canagliflozin.

Clinical Trial Registration Number: NCT01081834

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Canagliflozin reduces both HbA_{1c} and body weight in patients with type 2 diabetes mellitus on background metformin

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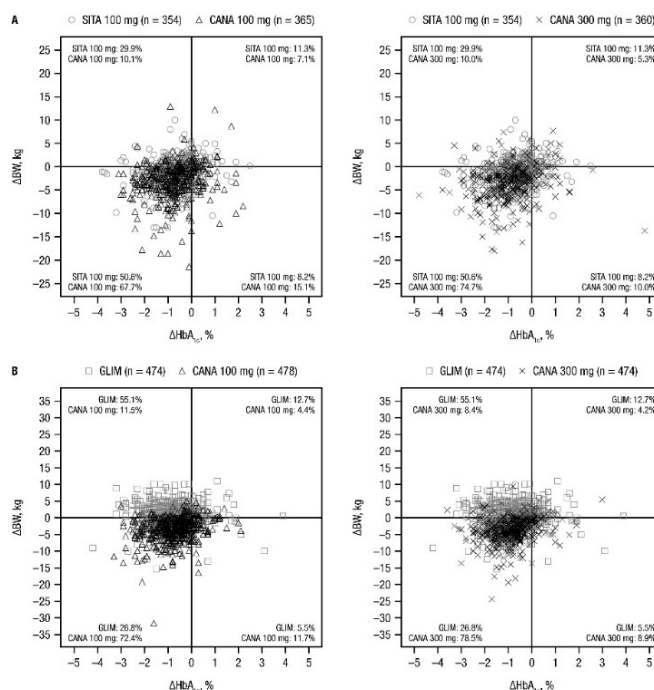
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Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, is approved for the treatment of adults with type 2 diabetes mellitus (T2DM). Reductions in HbA_{1c} and body weight (BW) have been observed with CANA in 2 studies as add-on to metformin over 52 weeks. This post hoc analysis describes the distribution of change from baseline in HbA_{1c} and BW in these studies at Week 52.

Materials and methods: This analysis used data from 2 separate randomised, double-blind, active-controlled Phase 3 studies of CANA 100 and 300 mg versus either sitagliptin (SITA) 100 mg (Study 1: N = 1,284; HbA_{1c}, 7.9%; BW, 87.2 kg) or glimepiride (GLIM; Study 2: N = 1,450; HbA_{1c}, 7.8%; BW, 86.6 kg). Study 1 included a treatment arm where patients received placebo during the 26-week core period and switched to SITA during the 26-week extension period (n = 183); this arm was excluded from the current analysis. **Results:** In Study 1, least squares (LS) mean HbA_{1c} changes from baseline were -0.73%, -0.88%, and -0.73%, and LS mean BW changes from baseline were -3.8%, -4.2%, and -1.3% with CANA 100 and 300 mg and SITA 100 mg, respectively. In Study 2, LS mean HbA_{1c} changes from baseline were -0.82%, -0.93%, and -0.81%, and LS mean BW changes from baseline were -4.2%, -4.7%, and 1.0% with CANA 100 and 300 mg and GLIM, respectively. A greater proportion of patients had reductions in both HbA_{1c} and BW with CANA 100 and 300 mg compared with SITA 100 mg (67.7%, 74.7%, and 50.6%, respectively) or GLIM (72.4%, 78.5%, and 26.8%, respectively; Figure). More patients had a decrease in HbA_{1c} with either no change or an increase in BW with SITA or GLIM versus CANA 100 and 300 mg in both studies.

Conclusion: CANA provided reductions in both HbA_{1c} and BW versus SITA 100 mg or GLIM in most patients with T2DM as add-on to metformin at 52 weeks.

Figure. Change from baseline in HbA_{1c} and BW at Week 52 in individual patients with CANA 100 and 300 mg versus (A) SITA and (B) GLIM.



BW, body weight; CANA, canagliflozin; SITA, sitagliptin; GLIM, glimepiride.

Clinical Trial Registration Number: NCT01106677, NCT00968812

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Glycaemic efficacy of canagliflozin is largely independent of baseline beta cell function or insulin sensitivity

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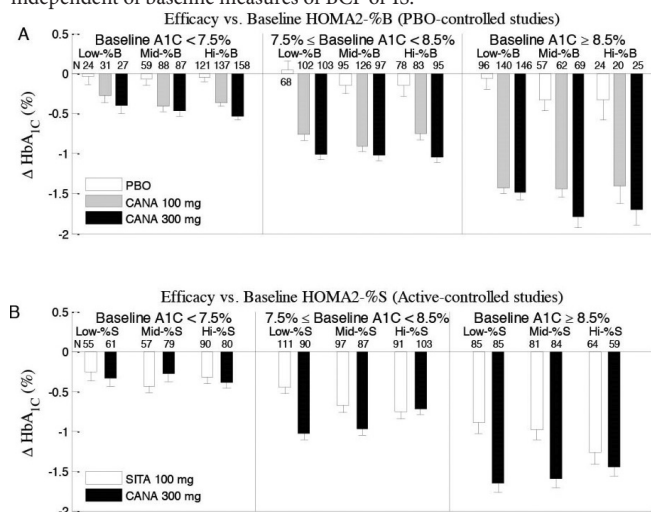
Background and aims: Canagliflozin (CANA), a sodium glucose co-transporter 2 (SGLT2) inhibitor, lowers plasma glucose and HbA_{1c} in patients with type 2 diabetes mellitus (T2DM) by increasing urinary glucose excretion. Because CANA's mechanism of action is insulin-independent, it was hypothesised that the glycaemic efficacy of CANA would be independent of baseline beta-cell function (BCF) or insulin sensitivity (IS). This hypothesis was tested using data from 4 placebo (PBO)- and 2 active-controlled studies to measure BCF (HOMA-%B) and IS (HOMA2-%S).

Materials and methods: The first analysis pooled data from 26-week PBO-controlled studies (N = 2,313; baseline HbA_{1c} = 8.0%, HOMA2-%B = 49, HOMA2-%S = 60). Patients were divided into tertiles of HOMA2-%B or HOMA2-%S and, given the potential impact of baseline HbA_{1c}, further divided based on baseline HbA_{1c} (HbA_{1c} <7.5%, 7.5% ≤ HbA_{1c} <8.5%, HbA_{1c} ≥8.5%). Change in HbA_{1c} from baseline with CANA 100 and 300 mg and PBO was evaluated for each subgroup. A second analysis included 52-week studies comparing CANA 300 mg with sitagliptin (SITA) 100 mg (N = 1,488; baseline HbA_{1c} = 8.0, HOMA2-%B = 49, HOMA2-%S = 59). Patients were divided into the same HOMA2-%B, HOMA2-%S, and baseline HbA_{1c} subgroups described in the first analysis.

Results: Overall, reductions in HbA_{1c} from baseline were greater in patients with higher baseline HbA_{1c} (Figures A and B). Within each baseline HbA_{1c} range, the HbA_{1c} decrease seen with CANA was generally similar across tertiles of HOMA2-%B (as shown for the PBO-controlled studies in Figure A; similar results for CANA were also observed in the active-controlled studies) and HOMA2-%S (as shown for the active-controlled studies in Figure B; similar results for CANA were also observed in the PBO-controlled studies). Greater reductions in HbA_{1c} were observed with CANA than with SITA,

with the biggest differences seen in patients with higher baseline HbA1c and in patients with lower HOMA2-%S (Figure B).

Conclusion: Consistent with expectations based on the insulin-independent mechanism of action of CANA, the glycaemic efficacy of CANA is largely independent of baseline measures of BCF or IS.



Supported by: Janssen Research & Development, LLC

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Effects of sodium glucose co-transporter 2 inhibitors in patients with type 2 diabetes: a systematic review with meta-analysis of randomised clinical trials

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Background and aims: Several randomised clinical trials (RCTs) have assessed the effects of sodium glucose co-transporter 2 inhibitors (SGLT-2i) for patients with type 2 diabetes. We performed a systematic review with meta-analyses of RCTs on SGLT-2i for ≥12 weeks. The primary outcomes were changes in HbA1c, body weight and adverse events.

Materials and methods: Random effects meta-analysis was performed. Bias and heterogeneity were assessed in subgroup, sensitivity, regression and sequential analyses. We included 24 placebo-controlled RCTs on 12–102-week treatment with canagliflozin 300 mg (7 trials), dapagliflozin 10 mg (13 trials) or empagliflozin 25 mg (4 trials) and 7 trials with active controls.

Results: Random effects meta-analysis of 3,425 patients randomised to SGLT-2i and 3,234 patients randomised to placebo found that SGLT-2i reduced HbA1c (mean difference -0.71%; CI -0.80 to -0.63) and body weight (-2.0 kg; -2.2, -1.9). The analysis was stable to assessments of bias and the results were confirmed in sequential analyses. Beneficial effects in favour of SGLT2i on fasting plasma glucose (-1.6 mM; CI -1.9 to -1.4), systolic blood pressure: (-4.4 mmHg; -5.2 to -3.6) and diastolic blood pressure: (-1.6 mmHg; CI -2.1 to -1.1) were detected. Analyses on adverse events showed that 6% of patients randomised to SGLT-2i developed genital or urinary tract infections (relative risk 1.7; CI 1.4 to 2.1); number needed to harm 50).

Conclusion: We conclude that SGLT-2i have clinically relevant effects on glycaemic control and body weight and should be considered as second line treatment after metformin in obese patients with type 2 diabetes. The risk benefit balance may not be favourable in patients prone to genital or urinary tract infections.

PS 066 Novel therapies

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Pharmacokinetics of once weekly dulaglutide in patients with type 2 diabetes mellitus

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Background and aims: The aim of these analyses was to characterize the pharmacokinetics (PK) of once weekly dulaglutide in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: PK meta-analysis of Phase 1 data and population PK modeling of Phase 2 and Phase 3 studies were used in order to perform this characterization.

Results: The PK of dulaglutide was well described by a 2-compartment model with first-order absorption. After subcutaneous (SC) administration, dulaglutide was slowly absorbed. Time to reach maximum concentration (C_{max}) at steady state ranged from 24 to 72 hours (median = 48 hours); absolute bioavailability was 47% and half-life was 4.7 days. Steady state was achieved between 2 and 4 weeks of dosing. At steady state for dulaglutide 1.5 mg, the mean peak (C_{max}) and total area under the concentration-time curve (AUC) exposures of dulaglutide were 114 ng/mL (range 56 to 231 ng/mL) and 14000 ng·h/mL (range 6940 to 26000 ng·h/mL), respectively; the accumulation ratio was approximately 1.56. Intra-patient variability was <17% for both AUC and C_{max} . Mean volume of distribution after intravenous administration was 5.32 L, showing dulaglutide distributes primarily in the blood volume. Apparent clearance in patients with T2DM after multiple 1.5 mg dosing was 0.107 L/hr. Body weight influenced dulaglutide PK, however weight explained <6% of the inter-patient variability. Effects of age, weight, sex, race and ethnicity on dulaglutide PK were not clinically relevant and generally less than the inter-patient variability of dulaglutide exposure (AUC, C_{max}) (≤35%). Injection site (abdomen, upper arm, thigh) had no statistically significant effect on exposure.

Conclusion: The PK of dulaglutide 1.5 mg supports once weekly administration in patients with T2DM. No dose adjustment is needed based on body weight, sex, age, race or ethnicity, or injection site.

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A multicentric, double-blind, randomised-controlled trial (RCT) of carnitine orotate complex in diabetic patients with non-alcoholic fatty liver disease (NAFLD)

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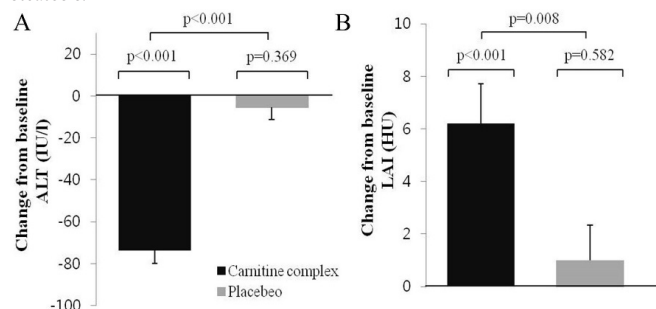
Background and aims: We aimed to evaluate the effects of carnitine orotate complex (Godex[®], 412mg/capsule, Celltrion Pharm, Seoul, Korea) on non-alcoholic fatty liver disease in diabetic patients. In addition, we also evaluated whether carnitine orotate have effect on the glucose metabolism, particularly, in relation to improvement of hepatic steatosis.

Materials and methods: Seventy-eight outpatients with NAFLD, who were receiving medical treatment for type 2 diabetes, were randomly assigned to receive double-blind oral carnitine orotate 900mg (GODEX[®]) daily (n=39) or placebo (n=39) for 3 months. Hepatic steatosis was assessed by using hepatic computed tomography (CT) attenuation values (Hounsfield units) obtained by unenhanced low-dose CT at baseline and 3 months after initiation of treatment. Also, biochemical marker for liver function and glycemic control were measured.

Results: There was no difference in the baseline mean liver attenuation between carnitine-treated and control groups. On the Hepatic CT analysis,

participants treated with carnitine orotate complex showed increase in mean liver attenuation values and liver attenuation index (LAI) after 12 weeks treatment ($p<0.008$ vs. placebo). Mean changes in LAI level from baseline at week 12 was 6.21 ± 8.51 in carnitine orotate complex group ($p<0.001$) while it was 0.74 ± 8.05 in placebo group ($p=0.582$). Both serum aspartate aminotransferase ($P<0.001$) and alanine aminotransferase ($P<0.001$) level were also reduced significantly in the carnitine-treated group. Those on treatment with carnitine showed improvement in HbA1c level with 0.33% decrease from baseline ($P<0.007$). When subjects were classified into tertiles by LAI changes from baseline at 12 weeks, participants in the highest tertile of LAI changes showed significant decrease in fasting glucose, HbA1c, and HOMA IR from baseline.

Conclusion: Three months of treatment with carnitine improved hepatic steatosis and liver enzymes levels in diabetic patients with NAFLD. Carnitine also improved glycemic control in relation to improvement of hepatic steatosis.



Clinical Trial Registration Number: KCT0000505

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Sulforaphane reduces hepatic glucose production in liver cells and improves glucose tolerance in diabetic animal models

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Background and aims: Novel means to treat type 2 diabetes are needed. One strategy for expanding the therapeutic options is drug repositioning, i.e. finding new indications for old drugs. The aim of this study was to employ a new method for drug repositioning combining gene network analysis of metabolic tissues and comprehensive public data on “drug signatures”. A drug signature denotes gene expression traits affected by a particular compound. Thus, we analysed gene co-expression networks in liver from different rodent models and identified a “disease signature” that predicts diabetes status. Next, we interrogated a library with >1000 drug signatures to identify drugs that can revert the aberrantly expressed genes in diabetes and improve metabolic control.

Materials and methods: A diabetes signature consisting of 50 genes was derived from rodent liver microarray data using co-expression analyses and causality tests. This signature was matched with publically available drug signatures using Gene Set Enrichment Analysis. The findings were validated by oral and intraperitoneal (i.p.) glucose tolerance tests (GTT) in Wistar rats and C57Bl6 mice with diet-induced glucose intolerance, as well as in clonal H-4-II-E liver cells.

Results: We identified sulforaphane (SFN) as the top candidate compound. SFN is an isothiocyanate contained in cruciferous vegetables. It is known to induce an anti-oxidant response by nuclear translocation of NRF2 and has recently been suggested to have anti-cancer effects and improve diabetic complications. First, we incubated hepatic H-4-II-E cells with 10 micromol/l SFN for 28 h. This decreased basal glucose output by 49% ($p=0.002$). Pre-incubation with palmitate (0.25 mmol/l) for 16 h elevated glucose output by 34% compared with non-treated control cells ($p=0.006$). Interestingly, SFN not only prevented the palmitate-induced increase of glucose production but led to a net reduction of glucose output by 45% compared with the control cells ($p=0.006$). SFN also improved the insulin-mediated inhibition of glucose production in these cells ($p=0.0003$). Next, Wistar rats with diet-induced insulin resistance were treated with 2.5 mg/kg SFN three times a week during 4 months, which improved insulin sensitivity as measured by an ip insulin tolerance test (AUC $p=0.0038$) and tended to decrease fasting glucose ($p=0.055$). Microarray analysis of liver from these rats revealed that a major fraction of the genes in the diabetic signature was reversed by SFN treatment ($p<0.0001$, Fisher's exact test). Treatment of rats with higher doses

of SFN (5 mg/kg daily) over shorter periods of time (2 weeks) improved glucose tolerance assessed by an oral GTT ($p=0.049$ at 60 min; AUC=0.093). Furthermore, mice with diet-induced diabetes demonstrated improved fasting glucose ($p=0.044$) and glucose tolerance measured by an i.p. GTT (AUC $p=0.011$) after 4-week treatment with 10 mg/kg SFN.

Conclusion: Through a new method for network-based drug repositioning we have identified SFN as a potential new anti-diabetic compound. Our results show that SFN improves diet-induced insulin resistance in vivo, reverses aberrantly expressed genes and reduces glucose output from H-4-II-E liver cells. The observed effects, in combination with the documented ability of SFN to protect tissues from diabetic complications and low toxicity, make SFN a promising new drug for future treatment of T2D.

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Timed daily bromocriptine mesylate (BC) administration improves glucose disposal in a canine diet-induced model of impaired glucose tolerance

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Background and aims: We previously showed that 4 wk of high-fat diet (HFD; 52% of energy) blunts net hepatic glucose (glc) uptake (NHGU) during a hyperinsulinemic hyperglycemic (HIHG) clamp in dogs. Here we examined the impact of BC treatment upon glucose disposal during an OGTT and a HIHG clamp in HFD-fed dogs.

Materials and methods: After an initial OGTT (0.9 g/kg), 10 dogs began the HFD. After 4 wk of HFD, 5 dogs received subcutaneous BC injections (15 µg/kg/d) and 5 (CTR) received vehicle for 26d (rx period) at the onset of locomotor activity, while continuing the HFD. Surgery for placement of hepatic balance catheters was performed ~16d before the end of rx. Dogs underwent OGTT2 ~5d before rx ended and a HIHG clamp (4x basal insulin, 2x basal glc) at the end of rx. During the 1st 90 min of the clamp (P1), glc was infused via peripheral vein; during the last 90 min (P2), glc was also infused via the hepatic portal vein at $22.2 \mu\text{mol}\times\text{kg}^{-1}\times\text{min}^{-1}$.

Results: By 4 wk of HFD, dogs had gained 4 ± 1 kg (mean \pm SEM); CTR and BC dogs lost 0.3 ± 0.2 and 0.9 ± 0.4 kg, respectively, during rx ($P=0.4$). In OGTT2 vs OGTT1, the $\Delta\text{AUC}_{0-120 \text{ min}}$ for glucose increased 58% (CTR) and 26% (BC; $P<0.05$ vs CTR), while that of insulin was increased 68% and 95% (CTR vs BC; $P=0.4$). The C-peptide response increased nearly 2-fold in CTR (OGTT2 vs OGTT1; $P<0.05$) but did not change in BC, suggesting that BC might decrease insulin clearance. In the clamp studies, there were no significant group differences during the basal or P1 periods. Nonhepatic glucose uptake was greater in BC vs CTR during P2, and total body glucose disposal (GIR) showed a strong tendency to be enhanced in BC (Table 1). There was no significant correlation between plasma insulin concentrations and net hepatic or nonhepatic glucose uptake.

Conclusion: BC rx of HFD-fed dogs improves glucose tolerance and glucose disposal during a HIHG clamp primarily by enhancing nonhepatic glucose disposal.

Table 1. Results of deep venous sampling during OGTTs 1 and 2

	Basal glc (mM)	Glc ΔAUC (mM*120 min)	Basal insulin (pM)	Insulin ΔAUC (pM*120 min)	Basal C-peptide (ng/ml)	C-peptide ΔAUC (ng/ml*120 min)
CTR - OGTT1	5.8 ± 0.2	164 ± 24	34 ± 8	8099 ± 1485	0.24 ± 0.05	55 ± 9
CTR - OGTT2	5.8 ± 0.2	259 ± 45	48 ± 9	13645 ± 2328	0.32 ± 0.08	123 ± 8
BC - OGTT1	6.0 ± 0.1	107 ± 28	34 ± 9	6409 ± 2052	0.25 ± 0.07	44 ± 14
BC - OGTT2	5.9 ± 0.1	135 ± 32 *	44 ± 6	12511 ± 2543	0.21 ± 0.05	50 ± 10 *

ΔAUC = change from basal values over the 1st 120 min after glc load. * $P<0.05$ vs CTR

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Prevention of diet-induced hepatic insulin resistance by antisense oligonucleotides targeted to mINDY

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Background and aims: INDY as part of the SLC13 protein family is a high-affinity di- and tricarboxylate plasma membrane transporter involved in citrate import. In Drosophila, genetic deletion of INDY alters energy metabolism and extends lifespan. Mice lacking INDY are protected from both

diet-induced and age-associated hepatic insulin resistance. Here, we examined the impact of selective hepatic knockdown of mammalian Indy protein (mINDY) expression using anti-sense oligonucleotides (ASOs).

Materials and methods: We studied the effect of mINDY knockdown on hepatic glucose metabolism in 4 week high fat fed rats (n=15 per group) assessed by hyperinsulinemic-euglycemic (HEC) clamp studies.

Results: After 4 weeks of ASO treatment, mINDY mRNA expression was reduced by 91% ($P<0.001$) in the treatment group. The mINDY ASO treated rats showed a 34% reduction in fasting plasma insulin concentrations compared to the control group (14.5 vs. 9.6 $\mu\text{U/ml}$, $P<0.05$) and was associated with ~30% reduction in basal rates of endogenous glucose production [5.9 ± 0.6 vs. 8.4 ± 0.8 mg/(kg·min), Furthermore hepatic insulin responsiveness was increased in the mINDY ASO rats as reflected by increased suppression of hepatic glucose production during the HEC [19.7 vs. 61.6% , $P<0.05$].

Conclusion: Taken together these data suggest that hepatic mINDY may be a novel therapeutic target for the treatment of hepatic insulin resistance and type 2 diabetes.

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LX4211 improves glycaemic control in NOD mice with type 1 diabetes

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Background and aims: There is a need for oral agents that improve glycemic control without increasing hypoglycemia (hypoG) events in patients with type 1 diabetes (T1D). LX4211 is a compound that may meet this need, as it lowers blood glucose (G) by the dual insulin (Ins)-independent mechanisms of inhibiting intestinal sodium-glucose cotransporter (SGLT)1 to delay G absorption and inhibiting kidney SGLT2 to decrease G reabsorption. We tested if LX4211 can improve the poor glycemic control of NOD mice receiving low daily insulin doses for their T1D, and if LX4211 treatment of these mice is accompanied by a decrease in hypoG events.

Materials and methods: NOD mice receiving only 0.05 U Ins/day for their T1D served as a model of poor glycemic control, while NOD mice receiving 0.2 U Ins/day for their T1D served as a model of improved glycemic control. T1D, defined as fed blood G > 300 mg/dL on 2 consecutive samples, was induced in 11 week old NOD mice using IP cyclophosphamide (200 mg/kg x2, separated by 2 weeks). Ins was delivered SC by Alzet pump (Model 1004, 4 week duration). The day after pump implantation, mice received the first of 22 once-daily oral doses of LX4211 or vehicle. Glycemic control was monitored by measuring blood A1C (A1C Now+ kit) and fed blood G (ACCU-CHEK Aviva glucometer). HypoG was defined as a fed blood G < 50 mg/dL.

Results: As is shown in the table below, 1) LX4211 significantly improved glycemic control in NOD mice with poorly controlled T1D, as evidenced by the rapid and sustained lowering of blood G levels, and the delayed rise in A1c levels, in LX4211-treated mice receiving 0.05 U Ins/day; 2) the glycemic control of LX4211-treated mice receiving 0.05 U Ins/day was similar to that of vehicle-treated mice receiving 0.2U Ins/day; and 3) hypoG was less frequent in LX4211-treated mice receiving 0.05 U Ins/day than in vehicle-treated mice receiving 0.2 U Ins/day.

Conclusion: LX4211 significantly improved glycemic control in NOD mice with poorly controlled T1D, and this improvement was associated with a marked decrease in the frequency of hypoG events.

Group			G			Δ A1C (%)	HypoG
Ins	LX4211		(mg/dL)				
N	(U)	(mg/kg)	Day -1	Day 2	Day 23		
10	0.05	0	511 \pm 62	471 \pm 69 *	498 \pm 125 ^	4.0 \pm 0.8 ^	0 / 100
10	0.05	2	514 \pm 75	303 \pm 92	306 \pm 149	2.1 \pm 0.9	0 / 100
9	0.05	30	507 \pm 80	231 \pm 105	225 \pm 131	1.4 \pm 0.9	0 / 90
10	0.2	0	516 \pm 80	257 \pm 135	256 \pm 156	1.8 \pm 1.3	5 / 100

Δ A1C = Δ A1C from baseline to Day 23; Δ A1C and G data are mean \pm SD.

HypoG = number of G < 50 mg/dL measurements / total G measurements.

N = number of mice. Different from all other groups, * $p < 0.05$; ^ $p < 0.01$

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Berberine increases serum osteocalcin levels and improves early phase of insulin secretion in non-alcoholic fatty liver disease patients with impaired glucose metabolism

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Background and aims: Increasing evidences have shown that osteocalcin participate in the glycemic control and energy metabolism. Positive modulation of serum osteocalcin concentrations could be valuable for the prevention, delay and treatment of diabetes. Berberine is the active phytochemical that has potential to treat diabetes and diabetic complications. We aim to observe the effect of berberine on serum osteocalcin levels in patients with non-alcoholic fatty liver disease (NAFLD) and impaired glucose metabolism. **Materials and methods:** We randomly assigned 124 adults with NAFLD and impaired glucose metabolism to 16 weeks of treatment with lifestyle intervention (LSI) (a reduction of 500 kcal in daily intake and exercise) or berberine (1.5g daily) plus LSI. Before and after treatment, we assessed serum glucose and lipid metabolism, insulin, osteocalcin levels, liver enzymes, and hepatic fat content by proton magnetic resonance spectroscopy.

Results: Berberine plus LSI treatment, as compared with LSI control, was associated with a significantly higher increasing in early phase insulin secretion ($\Delta\text{I30}/\Delta\text{G30}$) [6.47 ± 13.86 vs. -0.06 ± 10.56 , $p=0.011$] and serum osteocalcin levels [4.54 ± 4.55 vs. 0.85 ± 3.62 ug/L, $p<0.001$], and a significantly higher reduction in 2 hour blood glucose, HOMA-IR, total cholesterol, triglyceride, alanine aminotransferase, body weight, waist circumference and hepatic fat content (all $p<0.05$). Although these data had higher improvement in berberine plus LSI than in LSI group, such as fasting glucose, 30 minute glucose, HbA1c, LDL-c, aspartate aminotransferase and glutamyltransferase, the differences of them were insignificant. Spearman's correlation analysis demonstrated that the difference of osteocalcin before and after treatment was significantly positively associated with the difference of $\Delta\text{I30}/\Delta\text{G30}$, and negatively associated with the differences of 30 minute blood glucose, HbA1c, total cholesterol, BMI, waist circumference, and hepatic fat content before and after treatment in the two groups. A stepwise multiple linear regression analysis demonstrated that the difference of osteocalcin was the only variable independently associated with the difference of $\Delta\text{I30}/\Delta\text{G30}$ ($p=0.045$), the other variables excluded from the regression equation including differences of HbA1c, total cholesterol, triglyceride, HDL-c, LDL-c, BMI, waist circumference, WHR, blood pressure and hepatic fat content. The incidence of adverse events between groups were significantly different ($p<0.001$), with no serious adverse events occurred.

Conclusion: For NAFLD patients with abnormal glucose metabolism, Berberine can increase serum osteocalcin levels, improve early phase insulin secretion and decrease 2 hour blood glucose. Elevated levels of osteocalcin may be one of the mechanisms of berberine improving glucose metabolism.

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Proteinuric nephropathy in acquired and congenital generalised lipodystrophy: baseline and after recombinant leptin therapy

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Background and aims: Lipodystrophies, acquired and congenital, are syndromes characterized by absence of body fat, low levels of the adipocyte-derived hormone/cytokine leptin, severe hypertriglyceridemia, insulin resistance associated with diabetes, hepatomegaly and hepatic steatosis. Nephropathy has been previously reported but it is not a feature of the syndrome, but proteinuria was common and very high in this group of young patients. Aim: Evaluate basal renal disease in patients with lipodystrophy and modifications 3 and 6 months after r-metHuLeptin therapy.

Materials and methods: We studied 6 patients with lipodystrophy, 4 generalized (2 congenital generalized lipodystrophy CGL, and 2 acquired generalized lipodystrophy, AGL) and the other 2 with familial partial lipodystrophy (FPL). We determined basal, and after 3 and 6 month of leptin therapy: Creatinine, urea, microalbuminuria, proteinuria and % HbA1c. We evaluate which patients were on Angiotensin-converting enzyme (ACE) inhibitors therapy. In 2 of the 6 patients renal biopsy was done. We performed percutaneous renal biopsies in 2 patients.

Results: HbA1c and proteinuria improved after leptin therapy. Patient 2 didn't develop proteinuria. Four patients have proteinuria at baseline and after leptin improved significantly (patients 3, 5 and 6) or even turned negative (patient 1). Patients 1,3 and 5 were on enalapril. Renal Biopsy: patient 1 has diagnosis of mesangial proliferative glomerulosclerosis and Patient 3 developed focal segmental glomerulosclerosis (FSGS)

Conclusion: Renal function was evaluated in a group of patients with lipodystrophy during the course of a therapeutic trial of recombinant human leptin. It was describe that in some cases treated with recombinant human leptin there was an exacerbation of their underlying renal disease. In our patients we demonstrated efficacy of leptin to improved renal disease.

Patients	Type	Hb A1c%						Urea mg/dl			Creatinine			Microalbuminuria			Proteinuria			Enalapril
	Of							mg/dl			Ug/min			Ug/min			mg/d			
	LD	b	3	6	b	3	6	b	3	6	b	3	6	b	3	6				
P 1	CGL	14	8.3	8.5	11.6	29	31	0.8	0.49	0.43	0	0	0	0	0	0	1736	0	0	20
P 2	AGL	10.9	5.3	5.2	10	38	16	0.22	0.5	0.4	0	0	0	0	0	0	0	0	0	no
P 3	CGL	8	4.6	5.5	37	38	33	0.8	0.63	0.57	0	122	96	2423	0	0	0	0	10	
P 4	AGL	12.4	8.7	SD	SD	28	26	0.39	0.48	0.52	53	36.5	89.4	0	0	0	0	0	no	
P 5	FPL	9.5	ND	ND	17	29	0.3	ND	0.63	0	ND	38.3	221.8	0	0	0	0	0	2.5	
P 6	FPL	ND	6.6	ND	7	7	ND	0.3	0.5	ND	0	14.6	ND	205	0	ND	no	no	no	

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Ranolazine induces skeletal muscle hypertrophy activating Ca²⁺/calmodulin pathway

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Background and aims: The underlying causes of type 2 diabetes are a combination of a defect in insulin secretion from pancreatic β cells and an impairment in insulin-mediated glucose disposal (insulin resistance) in insulin target tissues, such as skeletal muscle. Ranolazine (RAN) is a novel anti-ischemic and antianginal drug that reduces angina frequency in patients with chronic stable angina, a manifestation of coronary artery disease. RAN decreases myocardial ischemia by improving sodium-calcium homeostasis via inhibition of the late phase of the inward sodium current (late I_{Na}). Recently, RAN has been shown to lower haemoglobin A1c (HbA1c) in patients with diabetes mellitus. The mechanism by which RAN improves glycaemic control is unknown. Previous studies in isolated human and murine β cell showed that RAN preserves pancreatic cells mass and promotes glucose-stimulated insulin secretion. However, RAN hypoglycaemic mechanisms in skeletal muscle is jet not studied. Objective of the present study was to determine the effect of RAN on skeletal muscle metabolism and differentiation.

Materials and methods: We examined RAN action on skeletal muscle using C2C12 murine myoblastic cell. After treatment of C2C12 with 1, 10 or 25 μ M RAN for 24 hours to study the RAN dose-response relationship: 10 μ M RAN was considered the dose able to stimulate morphological changes and hypertrophic process in neo-formed myotubes. 10 μ M RAN was added to C2C12 during proliferation, differentiation and neo formed myotubes to assess RAN possible interference in insulin pathway.

Results: RAN enhanced myoblasts proliferative capacity decreasing the expression of cell cycle inhibitors (p21 protein/cyclins). Interestingly, RAN did not modify p70S6 kinase activation showing it did not modulate the classic signaling pathways involved in cell growth. Western and Immunofluorescence studies revealed that during differentiation, RAN improved neo myotubes formation, but did not stimulate kinases involved in skeletal muscle differentiation and glucose uptake (ERKs and AKT pathways). Neo formed myotubes analysis showed that RAN activated Ca²⁺/calmodulin dependent protein kinase (CaMKII), which plays an important role in GLUT4 upregula-

tion and in myotube differentiation, confirming that RAN improved skeletal muscle metabolism a without affecting p70S6 and ERKs pathway.

Conclusion: Taken together, our results demonstrate that RAN have a positive action on skeletal muscle cells differentiation and metabolism, activating not AKT insulin mediated pathway, but Ca²⁺/calmodulin signaling pathway. This finding provides interesting evidence on the innovative use of RAN in diabetic condition, not only to improve insulin secretion of β cell but also insulin sensitivity in skeletal muscle.

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Lipolytic and insulinotropic effects of HM12525A, a novel long-acting GLP-1/glucagon dual agonist

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Background and aims: Oxyntomodulin, an alternative cleavage product of proglucagon, is a gut hormone which shows enhanced body weight loss and improved glycemic control by activating GLP-1 (GLP-1R) and glucagon receptor (GCGR), respectively. But its clinical application is limited due to a short half-life. We have developed the long acting GLP-1/glucagon dual agonist, HM12525A, by conjugating a novel GLP-1/glucagon dual agonist with constant region of human immunoglobulin via non-peptidyl linker. In a previous study, we demonstrated that once weekly administration of HM12525A exerted potent body weight loss and improved glycemic control in obese and diabetic animal models. However, the underlying modes of action for HM12525A are still poorly defined. The aim of this study was to investigate the molecular basis for the beneficial effects of HM12525A in vitro and in vivo system.

Materials and methods: To evaluate the effect of HM12525A on lipid droplet formation, 3T3-L1 cells were incubated with HM12525A during adipogenic differentiation. After 10–14days, the cells were stained with Oil-red O to determine triglyceride content. Fully differentiated 3T3-L1 adipocytes were treated with HM12525A, and the phosphorylation of hormone-sensitive lipase (HSL) was evaluated through western blot analysis. To measure lipolytic activity, conditioned medium was collected following HM12525A treatment for 4hr, and the amount of glycerol was quantified. Serum concentration of insulin in normal mice during ipGTT was quantified to evaluate the insulinotropic and insulin sensitizing potency of HM12525A.

Results: Consistent with *in vivo* results in which HM12525A administration significantly reduced the fat mass of diet-induced obesity (DIO) mice, HM12525A inhibited the lipid droplet formation in 3T3-L1 adipocytes in a dose-dependent manner. Of note, these inhibitory effects were partially reversed by either GLP-1R or GCGR antagonist, suggesting that the dual agonism of HM12525A synergistically exerts its lipolytic action. At the molecular level, phosphorylation of HSL, a key marker for the activation of lipolysis, and the following glycerol release were increased upon HM12525A treatment in 3T3-L1 adipocytes. As to the insulinotropic effects in pancreatic β -cells, HM12525A increased insulin secretion in RINm5F cells. In line with *in vitro* results, HM12525A administration significantly increased insulin secretion as well as insulin sensitivity, thereby attenuating the glucose excursion during ipGTT in normal mice.

Conclusion: Our results indicate that a well balanced dual agonism of HM12525A mediates synergistic effects on lipolysis of adipocytes. Moreover, HM12525A improves glucose tolerance by enhancing both insulin secretion and insulin sensitivity in β -cells and normal mice. Therefore, our results collectively demonstrate that a novel GLP-1/glucagon dual agonist HM12525A mediated lipolytic and insulinotropic effects through which anti-obesity and anti-diabetic potentials were conferred.

PS 067 Non-glycaemic effects of DPP4 inhibitors

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Renoprotective effect of gemigliptin, dipeptidyl peptidase 4 inhibitor, on streptozotocin-induced type 1 diabetic model

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Background and aims: Gemigliptin is a dipeptidyl peptidase 4 (DPP4) inhibitor, which is currently used in the treatment of patients with type 2 diabetes through augmentation of GLP-1 activity. In addition to GLP-1 activity, growing body of evidence shows that DPP4 play various roles in metabolism and inflammation through enzymatic activity and non-enzymatic activity. Some studies reported that DPP 4 activity increased in the urine and kidney of the diabetic patients and inhibition of DPP 4 activity attenuated diabetic neuropathy, retinopathy and renal ischemia-reperfusion injury. Therefore, the aim of this study was to determine whether gemigliptin has renoprotective effects on the kidney of streptozotocin (STZ)-induced type 1 diabetic mice model.

Materials and methods: Diabetes was induced by single intraperitoneal injection of streptozotocin (150 mg/kg/body weight). Diabetic mice was treated without or with an oral dose of gemigliptin 300mg/kg/ day for 8 weeks. Renal injury was observed by electron microscopy and light microscopy. We also measured serum glucose and urinary albumin excretion and evaluated fibrotic markers using immunohistochemical staining, qRT-PCR and western blot analysis.

Results: Blood glucose was significantly higher in diabetic mice than control mice, and gemigliptin did not reduce blood glucose levels of STZ-induced diabetic mice. Diabetic mice exhibited marked increased kidney/body weight ratio and urinary albumin excretion, but gemigliptin treatment significantly reduced kidney/body weight ratio and albuminuria. Moreover, gemigliptin treatment significantly reduced glomerular basement membrane thickness of kidney compared with STZ-induced diabetic mice. Histological examination showed renal fibrosis was induced by STZ, but gemigliptin treatment significantly attenuated STZ-induced renal fibrosis. In addition, immunohistochemical staining showed decrease of type 1 collagen and fibronectin in the kidney of STZ-induced diabetic mice treated with gemigliptin. To test whether attenuation of renal fibrosis by gemigliptine is GLP-1 dependent, we examined the effects of gemigliptin on TGF- β -stimulated fibrotic gene expression in cultured renal cells. The results showed that gemigliptin-treated RMCs and NRK-52E cells showed markedly decrease in type 1 collagen and fibronectin expression through inhibition of smad3 activity.

Conclusion: In conclusion, our data showed that gemigliptin has renoprotective effect on diabetic nephropathy regardless of glucose lowering effect. The present study raises the possibility that gemigliptin could be used to prevent the progress of diabetic nephropathy including patients with type 1 diabetes.

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Pituitary adenylate cyclase-activating polypeptide, a substrate of DPP-4, protects glomerular podocytes from inflammatory injuries

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Background and aims: Diabetic nephropathy (DN) is a leading cause of end-stage kidney disease; however, to date, there are few available treatment options. Although the mechanisms of DN are not well understood, inflammation plays a crucial role in the initiation and/or progression of DN. Dipeptidyl peptidase-4 inhibitors (DPP4i) have recently been introduced for use as oral hypoglycemic agents. In addition to reducing blood glucose levels, DPP4i have been found to possess pleiotropic actions, e.g. protecting the

kidney from injuries. Although the mechanisms underlying the pleiotropic effects of DPP4i are not clear, it has been speculated that substrates, including incretins, which are stabilized by DPP4i may play a role in these effects. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide, which was originally isolated from the ovine hypothalamus and has diverse biological functions. PACAP is known to be one of the substrates of DPP4. Furthermore, in studies of kidney pathology, PACAP has been found to have renoprotective effects. However, the specific cell types within the kidney that are protected by PACAP have not yet been identified. In the present study, we investigated the effects of PACAP on kidney cells and the mechanisms by which PACAP decreases the expression of inflammatory cytokines.

Materials and methods: We used immunohistochemistry (IHC), western blotting (WB), and real-time polymerase chain reaction (RT-PCR) to evaluate the expressions of PACAP receptors in kidney tissue, isolated glomeruli and cultured podocytes. Next, we evaluated if PACAP induces the phosphorylation of cAMP response element-binding protein (CREB) in cultured podocytes using WB. In addition, podocytes were stimulated with lipopolysaccharide (LPS) and PACAP, and the effects of PACAP on the expression of inflammatory cytokines were examined using WB or RT-PCR. Activation of NF- κ B and ERK were evaluated by nuclear localization of NF- κ B and phosphorylation of ERK, respectively.

Results: We found that VPAC1, one of the PACAP receptors, is expressed in podocytes, a key player of glomerular filtration barrier. PACAP (10 nM) significantly increased the cellular contents of cAMP in cultured podocytes. In the presence of PACAP, CREB was increased by 1.9 ± 0.5 fold (mean \pm SEM). In cultured podocytes, LPS increased the expression of interleukin-6 (IL-6) and monocyte chemo-attractant protein-1 (MCP-1) by 4.2 ± 0.6 fold and 32.8 ± 3.3 fold, respectively. In the presence of PACAP, the increased expression of IL-6 and MCP-1 was significantly attenuated by 20% and 25%, respectively, through the protein kinase A signaling pathway. In the presence of LPS, NF- κ B transnuclear localization and phosphorylation of ERK were significantly increased, and PACAP significantly reduced these increases by 51% and 81%, respectively.

Conclusion: In the present study we demonstrated that PACAP has anti-inflammatory effects on glomerular podocytes. To date, treatment options for DN are limited and PACAP may be useful in the prevention/attenuation of DN.

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Effect of two years of sitagliptin treatment on renal function in elderly patients with type 2 diabetes mellitus

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Background and aims: Many elderly patients with type 2 diabetes suffer from complications of chronic kidney disease and diabetic nephropathy. In this study, we examined the effect of a DPP-4 inhibitor, sitagliptin, on renal function in elderly patients with type 2 diabetes.

Materials and methods: A total of 70 patients aged ≥ 65 years with type 2 diabetes mellitus, which included 42 males (60%), were examined. Patients had the following characteristics: age (mean \pm SD) was 74 ± 5 years, BMI 24.5 ± 3.5 kg/m², duration of diabetes 14 ± 10 years, HbA1c $8.3 \pm 1.0\%$, serum creatinine 0.82 ± 0.27 mg/dl, eGFR 69.3 ± 23.9 ml/min/1.73m², and a urine albumin-to-creatinine ratio (UACR) of 94.1 ± 172.0 mg/gCr. Patients had been treated with either 25 mg (n = 6 [9%]) or 50 mg (n = 64 [91%]) of sitagliptin over a two-year period. HbA1c, eGFR and UACR were assessed yearly. eGFR data had been collected 1 year before commencement of sitagliptin. Of the 70 patients, 22 (31%) had eGFR < 60 ml/min/m² and 28 (40%) had UACR > 30 mg/gCr. All endpoints were evaluated by ANOVA.

Results: HbA1c values before treatment, 1 year, and 2 years after sitagliptin treatment were 8.3, 7.5, and 7.3, respectively (before vs. after treatment, $p < 0.0001$). Gradually decreasing eGFR values were noted, as evidenced by values of 71.0, 69.3 ($p < 0.05$ when compared with eGFR 1 year before treatment), 65.6 ($p < 0.05$ when compared with eGFR before treatment), and 63.0 ($p < 0.005$ when compared with eGFR before treatment) at 1 year before treatment, before treatment, 1 year, and 2 years after treatment, respectively, while eGFR values were not decreased in patients with reduced renal function (eGFR < 60 ml/min/m²), as evidenced by values of 52.5, 47.6 ($p < 0.05$ when compared with eGFR 1 year before treatment), 47.8 ($p = 0.88$ when compared with eGFR before treatment), and 45.5 ($p = 0.54$ when compared with eGFR before treatment), respectively. UACR values were significantly decreased in patients with micro- or macroalbuminuria (UACR > 30 mg/gCr), as evidenced by values of 207.3, 136.5 ($p = 0.27$ when compared with

UACR before treatment), and 106.6 ($p < 0.05$ when compared with UACR before treatment) before treatment, 1 year, and 2 years after treatment, respectively.

Conclusion: Despite the gradual decline in eGFR values in patients with normal renal function, eGFR did not decrease in patients with reduced renal function. These findings suggest that sitagliptin exhibits a renal protective effect, and is effective in maintaining long-term glycemic control in elderly patients with type 2 diabetes.

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A DPP-4 inhibitor suppresses atherosclerotic lesions in the aorta and coronary arteries with decrease of macrophage infiltration in cholesterol-fed rabbits

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Background and aims: Several studies have demonstrated suppression of atherosclerosis by dipeptidyl peptidase-4 (DPP-4) inhibitors in hypercholesterolemic mice. However, it remains unknown whether DPP-4 inhibitors might also exert anti-atherogenic effects in bigger animals. We examined the effect of anagliptin, a DPP-4 inhibitor, on the development of atherosclerosis in the aorta and coronary arteries in cholesterol-fed rabbits.

Materials and methods: Japanese white rabbits were fed either a normal diet or a diet containing 0.5% cholesterol. The cholesterol-fed rabbits were given drinking water not mixed ($n = 18$) or mixed with 3 mg/mL of anagliptin ($n = 16$) for 12 weeks. The lipoprotein fractions were measured by high-performance liquid chromatography. The serum oxidative stress markers 8-hydroxy-2'-deoxyguanosine (OHdG) and malondialdehyde (MDA) were measured by ELISA and TBARS, respectively. Inflammatory cytokine gene expressions in the carotid artery were quantified by real time-qPCR. We measured the lesion area in the aorta and the coronary arteries in seven cross-sections of the heart. Values were expressed as mean \pm SE.

Results: Dietary cholesterol intake led to a marked increase of the serum total-cholesterol (TC) level (37.9 ± 3.9 vs. 0.59 ± 0.04 mmol/L), with the most striking increase seen in the VLDL fraction (30–80 nm) among the major lipoproteins. No significant changes of the body weight, water intake, HbA1c, serum lipids and lipoproteins, or glucose response to intravenous glucose loading were observed following the administration of anagliptin. The plasma DPP-4 activity was suppressed by 86%, and the plasma active GLP-1 levels doubled. Dietary cholesterol intake resulted in the development of severe atherosclerosis in the aorta, with a ratio of the lesion area to the total aortic surface areas of $22.0 \pm 2.3\%$, and anagliptin treatment induced marked suppression of the percent lesion area to $8.6 \pm 2.1\%$ ($p < 0.001$). Atherosclerotic lesions were also observed in the coronary arteries. The four major coronary arteries increased in area with cholesterol feeding (1.14 ± 0.12 vs. 0.81 ± 0.06 mm²), while anagliptin treatment attenuated this change (0.87 ± 0.07 mm²). The intimal formation in the coronary arteries in the cholesterol-fed mice was attenuated by anagliptin treatment (0.04 ± 0.02 vs. 0.13 ± 0.05 mm²). The alpha-SMA-positive and macrophage-positive areas in the coronary arteries were suppressed 54 and 78% respectively, by anagliptin treatment ($p < 0.05$). Notably, the ratio of the macrophage area to the plaque area was substantially decreased by 83%. The serum 8-OHdG and MDA showed no significant changes following the anagliptin treatment. However, the arterial gene expressions of the inflammatory cytokines TNF-alpha and interleukin-6 were markedly reduced by approximately 90% ($p < 0.001$ – 0.05), and the expressions of macrophage chemoattractant protein-1 and CD26 in the carotid arteries were partially reduced (by about 30%) following the anagliptin administration.

Conclusion: Our study demonstrated for the first time that a DPP-4 inhibitor suppressed the development of atherosclerosis in the aorta and coronary arteries in bigger animals than rodents, by inhibiting the inflammatory responses in the vessels.

Supported by: Sanwa Kagaku

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A DPP-4 inhibitor remarkably suppresses foam cell formation in peritoneal macrophages obtained from db/db diabetic mice, comparison with a SGLT2 inhibitor and pioglitazone

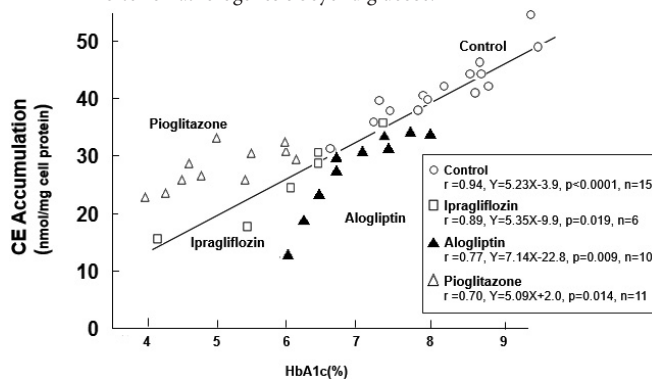
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Background and aims: We reported that a dipeptidyl peptidase-4 inhibitor (DPP-4i) confer an anti-atherosclerotic effect in diabetic apolipoprotein-E null mice with significant suppression of foam cell formation in macrophages (Mφ). However, it remains to be elucidated whether a DPP-4i exerts anti-atherogenic properties beyond glycaemic control. We evaluated the suppressive effect of DPP-4i on foam cell formation in Mφ obtained from db/db diabetic mice, and compared with other glucose-lowering agents, a sodium-glucose co-transporter (SGLT)2-inhibitor and pioglitazone.

Materials and methods: Male db/db mice at 9-week-old were fed normal diet containing none ($n=23$), alogliptin (0.02% w/w, $n=12$), pioglitazone (0.02%, $n=12$), or ipragliflozin (0.0014%, $n=13$) for 4 weeks. In subset of animals at age of 13 weeks, glucose (0.5g/kg body weight) was administered orally, and bled 0, 15, 30, 60, and 120 min thereafter (OGTT). Peritoneal Mφ were obtained from mice at age of 13 weeks after the injection of thioglycollate. Foam cell formation was determined by the incorporation of [³H]-oleate into cholesteryl-oleate stimulated by oxidized-LDL in Mφ. CD36 and Acetyl-CoA acetyltransferase (ACAT)-1 gene expression was measured by RT-PCR.

Results: Food and water intakes were comparable among treated groups, whereas final body weight was increased in pioglitazone group by 6% and decreased in ipragliflozin group by 5% compared with non-treated control group. Pioglitazone and ipragliflozin decreased fasting blood glucose (FBG), HbA1c and glucose-AUC in OGTT compared with control group (FBG: 6.1 ± 0.4 , 10.0 ± 1.6 , and 19.4 ± 1.8 mmol/l, HbA1c: 5.2 ± 0.2 , 5.7 ± 0.3 , and $8.4 \pm 0.2\%$, AUC: 790 ± 280 , 2123 ± 260 , 4183 ± 293 mmol/l \times min, respectively). Alogliptin group exhibited mild reductions of FBG, HbA1c, and AUC (16.0 ± 1.3 mmol/l, $7.0 \pm 0.4\%$, and 3413 ± 463 mmol/l \times min). Despite mild amelioration of glycemic control with alogliptin, the Mφ foam cell formation was 36% decreased, which was comparable to those with pioglitazone or ipragliflozin (35 and 30%, respectively). Foam cell formation was critically regulated by glycemic control (HbA1c) in each group ($r=0.70$ – 0.94 , $p<0.02$). The linear regression curves were similar between control and ipragliflozin groups. Alogliptin group distributed below the regression curves made by control and ipragliflozin groups, indicating that foam cell formation of alogliptin group is further suppressed than corresponded HbA1c. Gene expressions of CD36 and ACAT-1 were upregulated in db/db mice. Alogliptin treatment decreased gene expression of CD36 and ACAT-1.

Conclusion: A pioglitazone and a SGLT2-inhibitor both suppress foam cell formation in obese diabetic mice by substantial glucose-lowering effect, whereas DPP-4inhibitor remarkably suppresses foam cell formation despite mild amelioration of glycaemic control, suggesting a pleiotropic effect of DPP-4 inhibitor on atherogenesis beyond glucose.



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Increase in beta cell function and improvement in HOMA-2beta index induced by saxagliptin in patients with latent autoimmune diabetes in adultsR. Buzzetti¹, P. Pozzilli², R. Frederick³, N. Iqbal³, B. Hirshberg⁴;¹Department of Experimental Medicine, Sapienza University of Rome,²Department of Endocrinology and Diabetology, Policlinico Universitario Campus Bio-Medico, Rome, Italy, ³Bristol-Myers Squibb, Princeton,⁴AstraZeneca, Wilmington, USA.

Background and aims: We used the presence of glutamic acid decarboxylase antibodies (GADA) to identify patients with latent autoimmune diabetes of adults, a population which typically responds poorly to oral antidiabetic medications. We previously reported that saxagliptin (SAXA; 2.5, 5, and 10 mg/d) was generally well tolerated and produced greater reductions vs placebo (PBO) in HbA_{1c} and fasting and postprandial plasma glucose, with consistent treatment effects in GADA-positive and GADA-negative patients.

Materials and methods: In this analysis, we assessed the effects of SAXA on β -cell function (postprandial C-peptide AUC and HOMA-2 β) in GADA-positive (n=133) and GADA-negative (n=2576) patients from 5 placebo-controlled clinical trials.

Results: There were little or no changes from baseline to week 24 in fasting C-peptide concentrations across patient and treatment groups (Table). SAXA produced similar increases relative to PBO in C-peptide AUC and HOMA2- β in GADA-positive and GADA-negative patients, with no evidence of an interaction of GADA status on treatment effects, although the number of GADA-positive patients limits this interpretation.

Conclusion: These results suggest that SAXA improves β -cell function over 24 weeks in patients with and without GADA at baseline.

	GADA Positive		GADA Negative	
	SAXA	PBO	SAXA	PBO
Fasting C-Peptide, ng/mL				
Treatment-by-subgroup interaction <i>P</i> =0.27				
n	98	35	1822	708
Baseline	3.1 (0.13)	3.8 (0.27)	3.4 (0.03)	3.3 (0.05)
Mean change (95% CI) from baseline	0.1 (−0.12, 0.32)	−0.1 (−0.46, 0.28)	0.1 (0.08, 0.18)	0.2 (0.10, 0.27)
Difference (95% CI) vs PBO	0.19 (−0.24, 0.62)		−0.06 (−0.15, 0.04)	
Postprandial C-Peptide AUC, ng·min/mL				
Treatment-by-subgroup interaction <i>P</i> =0.42				
n	60	21	1300	489
Baseline	1013 (53.4)	1211 (95.5)	1107 (12.0)	1065 (19.8)
Mean change (95% CI) from baseline	124 (42.1, 206.1)	54.6 (−84.1, 193.2)	191 (173.0, 209.6)	54.5 (24.9, 84.1)
Difference (95% CI) vs PBO	70 (−91.4, 230.5)		137 (102.9, 170.7)	
HOMA2-β, %				
Treatment-by-subgroup interaction <i>P</i> =0.60				
n	96	33	1754	685
Baseline	59 (2.9)	70 (6.1)	68 (0.9)	66 (1.4)
Mean change (95% CI) from baseline	8.4 (1.4, 15.4)	−2.7 (−14.6, 9.3)	12.8 (11.1, 14.5)	5.5 (2.8, 8.2)
Difference (95% CI) vs PBO	11.1 (−2.8, 24.9)		7.3 (4.2, 10.4)	
Data are mean (SE) unless otherwise noted. Data were adjusted for baseline values and analyzed by ANCOVA, with terms for study, treatment, subgroup, treatment by subgroup, and baseline covariate. LOCF was used for missing data.				

Data are mean (SE) unless otherwise noted. Data were adjusted for baseline values and analyzed by ANCOVA, with terms for study, treatment, subgroup, treatment by subgroup, and baseline covariate. LOCF was used for missing data.

Clinical Trial Registration Number: NCT00121641, NCT00316082, NCT00121667, NCT00295633, NCT00313313

Supported by: BMS and AZ

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Efficacy of DPP-4 inhibitors and their effect on blood cyclosporine levels in patients with post-transplantation diabetesE. Kang¹, J. Bae¹, Y. Lee¹, C. Ahn¹, B. Cha¹, H. Lee¹, K. Huh², M. Kim², Y. Kim²;¹Internal Medicine, ²Transplantation Surgery, Yonsei University Health System, Seoul, Republic of Korea.

Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors have been widely used in kidney-transplant patients with diabetes. However the efficacy and interactions with immunosuppressants according to individual DPP-4 inhibitors are not widely studied. Therefore we tried to compare the glucose-lowering efficacy of DPP-4 inhibitors in patients with post-transplantation diabetes and evaluate the drug interaction with immunosuppressant, cyclosporine.

Materials and methods: A total of 91 renal allograft recipients with diabetes who began to take DPP-4 inhibitors after transplantation were enrolled. The glucose-lowering efficacy of three DPP-4 inhibitors, vildagliptin, sitagliptin, and linagliptin were compared using glycosylated hemoglobin (HbA_{1c}) after treatment for three months. Changes in blood cyclosporine levels were also assessed in 48 patients treated with cyclosporine.

Results: There were no significant differences in HbA_{1c} levels among vildagliptin, sitagliptin, and linagliptin treatment (−0.29±1.71% vs. −0.40±1.87% vs. −0.82±1.32%, P=0.559). However, blood cyclosporine level was significantly increased in the sitagliptin group, compared with vildagliptin and linagliptin group after three months of treatment (P=0.005). Difference in cyclosporine blood level was significant between vildagliptin group and sitagliptin group (P=0.004).

Conclusion: There were no significant difference in glucose-lowering efficacy among vildagliptin, sitagliptin, and linagliptin in kidney-transplant recipients with diabetes. However, drug interaction between cyclosporine was significant in patients with sitagliptin treated group. Physicians should consider drug interaction when prescribing sitagliptin in kidney-transplant patients receiving cyclosporine as an immunosuppressant.

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A comparison of the effects of the DPP-4 inhibitor sitagliptin and the sulfonylurea glimepiride on metabolic parameters and endothelial functionH. Nomoto¹, H. Miyoshi¹, A. Nakamura¹, T. Kondo¹, N. Manda², Y. Kurihara³, T. Atsumi¹, S. Aoki⁴;¹Medicine II (Immunology and Metabolism), Hokkaido University, ²Manda Memorial Hospital, ³Kurihara Clinic, Sapporo, ⁴Aoki Clinic, Sapporo, Japan.

Background and aims: DPP-4 inhibitors improve hyperglycemia in a glucose-dependent manner, and have been reported to possess favorable effects on atherosclerosis in animal experiments. However, it has not been elucidated whether DPP-4 inhibitors are able to improve endothelial function in patients with type 2 diabetes. Therefore, we investigated the efficacy of the DPP-4 inhibitor, Sitagliptin (Sita) on endothelial function and glycemic metabolism compared with Glimepiride (Gli) therapy.

Materials and methods: This study was a multicenter, prospective, randomized parallel-group comparison. Study inclusion criteria were current metformin treatment and inadequate glycemic control (HbA_{1c} levels of 6.9% to 8.4%) with sufficient control of blood pressure and lipid profile. Patients who suffered severe atherosclerosis, liver damage and renal dysfunction were excluded. Flow mediated dilation (FMD), Endo PAT, a comprehensive panel of hemodynamic parameters (Task Force® Monitor), and serum metabolic markers were assessed before and after the 26 week treatment period. All FMD assessments were performed by the same individual in a quiet, temperature-controlled setting. Patients were randomly assigned to once daily Sita (50mg) or Gli (0.5 to 2mg) therapy according to age, body mass index and baseline FMD values. Statistical analysis was performed using a Mann-Whitney U test and a p value < 0.05 was considered significant.

Results: Forty-six men and thirty-three women aged 59.0 ± 10.4 years with HbA_{1c} levels of 7.1 ± 0.4 % were enrolled. After 26 weeks, improvements in HbA_{1c} levels were similar between the Sita and the Gli group (p = 0.48). Contrary to expectations, the observed improvements in %FMD and Endo PAT were not statistically different between groups (%FMD; Sita 5.9 to 6.0 %, Gli 5.7 to 6.1 %, RHI; Sita 1.9 to 2.3, Gli 2.1 to 2.3). Plasma HDL-cholesterol, adiponectin and TNF- α levels were significantly improved in the Sita group (p < 0.05) and LDL-cholesterol also tended to be improved (p = 0.07). Blood

pressure, cardiac index, total peripheral resistance index and most other metabolic parameters were not different.

Conclusion: In type 2 diabetic patients without advanced atherosclerosis, changes in endothelial function were similar between Sita and Gli groups after 26 weeks. However, early Sita therapy was associated with more favorable effects on adipokine and lipid profiles compared with Gli therapy. Therefore, it is probable that over the long term these beneficial changes in glycemic and lipid control will aid in the prevention of atherosclerosis.

Clinical Trial Registration Number: UMIN000004955

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Co-administration of the DPP-4 inhibitor linagliptin and native GLP-1 induce synergistic body weight loss and appetite suppression in DIO rats
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Background and aims: Linagliptin is a dipeptidyl peptidase (DPP)-4 inhibitor approved for the treatment of type 2 diabetes. DPP-4 inhibitors are weight-neutral, suggesting that elevation of endogenous incretin (GLP-1) levels is not sufficient to promote weight loss *per se*. However, it may be possible that long-term exogenous GLP-1 treatment in the context of concurrent DPP-IV inhibition may yield synergistic effects on appetite and body weight regulation. Hence, we evaluated the chronic metabolic effects of linagliptin and native GLP-1(7-36) co-administration in DIO rats.

Materials and methods: Male diet-induced obese (DIO) rats (n= 8-10 per group) were treated with either linagliptin (1.5 mg/kg, PO, BID; 0.5 mg/kg, SC, BID) or native GLP-1 (0.4 mg/kg, SC, BID) as monotherapy, and compared to co-administration of linagliptin and GLP-1 for a total of 28 days. Body weight, food intake and body composition was measured. Also, forebrain preprodynorphin gene expression levels were analyzed to assess for potential central effects on central endogenous opioidergic neurotransmission.

Results: In DIO rats, monotherapy with linagliptin did not significantly influence Food intake, body weight and adiposity during the study period. In contrast, combined linagliptin and GLP-1 treatment induced a reduction in food intake in conjunction with a pronounced body-weight (~8% compared with baseline) and whole-body fat mass (~20% vehicle-corrected) lowering effect at study end, which was superior to GLP-1 administration *per se*. Notably, the anorexigenic effect of linagliptin and GLP-1 co-administration was associated with a marked increase in chow preference at the expense of palatable high-fat carbohydrate diet intake. Interestingly, combined linagliptin and GLP-1 treatment increased preprodynorphin mRNA levels in the caudate-putamen, an effect not obtained with administration of the compounds individually.

Conclusion: These data demonstrate that combined treatment with linagliptin and GLP-1 synergistically reduces body weight in obese rats. This anti-obesity effect is caused by appetite suppression and change in diet preference, presumably associated with increased dynorphin activity in dopaminergic forebrain regions involved in reward anticipation and habit learning. In conclusion, linagliptin and GLP-1 co-administration may therefore hold promise as a novel therapeutic principle for combined weight and diabetes management in obese patients.

Supported by: Boehringer Ingelheim

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Effects of sitagliptin on body fat and intrahepatic lipid content in Japanese overweight patients with type 2 diabetes

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Background and aims: Previous studies showed that human hepatocyte and adipocyte have the receptor of glucagon-like peptide-1 (GLP-1). Actually, GLP-1 analog, exendin-4 decreases intrahepatic lipid (IHL) and body fat in obese patients with type 2 diabetes. However, concrete changes of body fat and IHL by DPP-IV inhibitors were not fully evaluated. Thus, the aim of this study was to evaluate the effect of DPP-IV inhibitor, sitagliptin on body fat and IHL in overweight Japanese patients with type 2 diabetes.

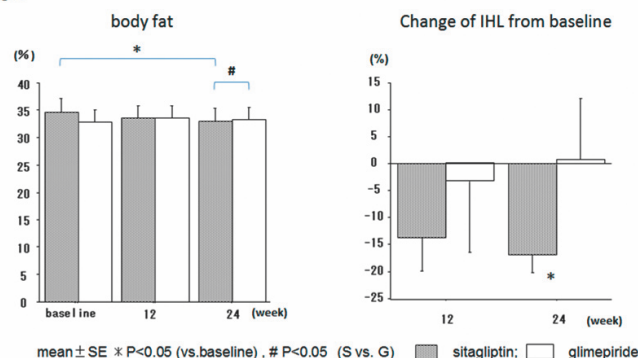
Materials and methods: This was a prospective, 24-week, single center, open labeled comparison study. The subjects were 20 Japanese type 2 diabetic patients (male: 11, female: 9) with BMI>25 kg/m². Nine of them were drug naïve

and the remaining 11 received metformin. After a 4 to 8 weeks period of instructing lifestyle modification (28 kcal/kg of ideal body weight and 150 - 200 kcal daily exercise), subjects were randomly assigned to receive sitagliptin 25mg titrated up to 50 mg (S) or glimepiride 0.5 mg titrated up to 1 mg (G). At the baseline, week 12 and week 24 after starting each treatment, body fat and IHL at segment 6 of the liver were evaluated by dual energy X-ray absorptiometry (DEXA) and ¹H-magnetic resonance spectroscopy (¹H-MRS), respectively.

Results: Though HbA1c and GA levels were significantly decreased in both groups after 24 weeks (HbA1c; 7.3 ± 0.1 to 6.5 ± 0.1% (S) and 7.1 ± 0.2 to 6.6 ± 0.1% (G), GA; 18.2 ± 1.0 to 14.8 ± 1.0% (S) and 17.0 ± 0.5 to 15.7 ± 0.4% (G), all p<0.05 vs. baseline), no significant differences were observed between the two groups (p=0.15). The body fat was not altered in G, but significantly decreased by 1.2% in S (p<0.05 vs. baseline). Reduction of IHL at week 24 was -0.8 ± 11.4% (p=0.47 vs. baseline) in G and 16.8 ± 3.5% (p<0.01) in S. No severe hypoglycemia event occurred in both groups during the study period.

Conclusion: Our findings indicate that while sitagliptin and glimepiride achieve blood glucose control to the same degree, the effects on adiposity were different: sitagliptin might have a beneficial effect on body fat and IHL apart from glucose control.

Fig1.



Clinical Trial Registration Number: UMIN00013356

PS 068 Incretin based therapies in special

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Efficacy and safety of saxagliptin in older participants in the SAVOR-TIMI 53 trial

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Background and aims: There are limited data from randomised clinical trials (RCT) on the efficacy and safety of antihyperglycaemic treatments in the elderly and very elderly despite the high prevalence of diabetes in these populations. The aim of this analysis was to examine the cardiovascular (CV) effects, glycaemic efficacy, and safety of saxagliptin (SAXA) in the elderly (≥ 65 years, N=8,561) and very elderly (≥ 75 years, N=2,330) participants in the SAVOR-TIMI 53 trial.

Materials and methods: Individuals ≥ 40 years old (N=16,492) with HbA_{1c} $\geq 6.5\%$ and $\leq 12.0\%$ were randomised (1:1) to double-blind saxagliptin (5mg or 2.5mg OD) or placebo treatment for a median follow-up of 2.1 years.

Results: The exposure to study medication was similar in all age groups. The hazard ratio (HR) for SAXA vs. placebo for the primary CV endpoint (myocardial infarction, ischemic stroke, CV death) was 0.92 for those ≥ 65 years vs. 1.15 for those < 65 years ($p=0.058$), and 0.95 for those ≥ 75 years (nominal interaction p -value, 0.67). The HR for the secondary composite end point and total mortality in the elderly and very elderly was balanced and showed a similar pattern to that of the entire study population. There was no treatment interaction based on age in terms of the risk of hospitalization for heart failure (p for interaction with age, 0.34), a component of the secondary end point, which showed an imbalance in the overall trial population. The difference in HbA_{1c} associated with the use of SAXA was comparable (-0.33% vs. -0.36%) in those \geq and < 75 years with a baseline HbA_{1c} of 7.6%. The incidence of overall adverse events (AEs), serious AEs and discontinuations related to serious AEs was similar in the elderly, very elderly and the entire study population between SAXA and placebo.

Conclusion: The SAVOR-TIMI 53 trial provides efficacy and safety data from a RCT with a robust number of elderly and very elderly participants. The efficacy, CV safety, and overall safety of saxagliptin in this subset of patients are similar to those found in younger patients supporting the safety of SAXA in the older patient population.

Clinical Trial Registration Number: NCT01107886

Supported by: AstraZeneca/Bristol-Myers Squibb

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Effects of baseline glycaemic HbA1c on CV outcomes and blood glucose control during the EXAMINE trial in patients with type 2 diabetes and recent acute coronary syndrome

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Background and aims: EXAMINE was a randomized, double-blind cardiovascular (CV) outcomes safety trial in patients with diabetes and recent acute coronary syndromes (ACS) that showed similar rates of major adverse CV events (MACE) with the dipeptidyl peptidase 4 (DPP-4) inhibitor alogliptin compared with placebo. In this analysis, we investigated the effects of baseline

HbA1c on MACE and the effect of alogliptin vs placebo on HbA1c according to baseline HbA1c levels.

Materials and methods: EXAMINE randomized 5380 patients in 49 countries with type 2 diabetes and an ACS within 15 to 90 days to alogliptin or placebo in addition to both evidence-based CV prophylaxis and treatment for type 2 diabetes according to regional guidelines. The median follow-up period in the trial was 18 months. Events were prospectively adjudicated by an independent, blinded committee (C5, Cleveland Clinic). The relationship between MACE and baseline HbA1c was analyzed using Cox proportional hazards models without adjustment for multiple comparisons.

Results: The HbA1c at the final visit was lower in the alogliptin arm vs placebo for each level of baseline HbA1c (Table). These changes occurred despite greater intensification of all additional medications in the placebo group. There was no systematic change in MACE rates according to baseline HbA1c (Table), $p = 0.971$ for interaction.

Conclusion: In patients with type 2 diabetes at high CV risk, there was no relationship between MACE and baseline HbA1c; the highest event rates were seen at an HbA1c of between 8 and 9%. Participants in the alogliptin arm achieved a lower HbA1c than those on placebo at all levels of baseline HbA1c. Of note, clinician investigators did not add sufficient additional therapy in those taking placebo to match HbA1c levels by the end of this double-blind randomized trial.

HbA1c, Baseline (%)	Mean Baseline, LS Mean Change in HbA1c from Baseline to Final Visit (n)*		LS Mean difference in HbA1c change from baseline to final visit [alogliptin vs. placebo]	95% CI
	Alogliptin	Placebo		
< 7	6.64%, 0.13% (n=454)	6.65%, 0.41% (n=464)	-0.28%	-0.42%, -0.14%
7 to < 8	7.45%, 0.10% (n=949)	7.44%, 0.30% (n=948)	-0.40%	-0.51%, -0.28%
8 to < 9	8.41%, -0.32% (n=768)	8.42%, -0.08% (n=726)	-0.23%	-0.39%, -0.08%
≥ 9	9.72%, -1.15% (n=529)	9.74%, -0.62% (n=541)	-0.54%	-0.74%, -0.34%
HbA1c, Baseline (%)	MACE Rates, (n)*		Hazard Ratio [†] [alogliptin vs. placebo]	95% CI [‡]
	Alogliptin	Placebo		
< 7	10.6% (n=454)	10.8% (n=464)	1.04	0.70, 1.54
7 to < 8	11.2% (n=949)	11.9% (n=948)	0.95	0.73, 1.24
8 to < 9	12.8% (n=768)	12.7% (n=726)	0.95	0.72, 1.26
≥ 9	10.0% (n=529)	11.3% (n=541)	0.89	0.62, 1.28

*Number of patients per baseline HbA1c subgroup. [†]Derived from Cox proportional hazards models with a factor for treatment and stratified by screening renal function and geographic region.

Clinical Trial Registration Number: NCT00968708

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Efficacy of vildagliptin and sitagliptin in patients with type 2 diabetes and severe renal impairment

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Background and aims: There are limited clinical data comparing DPP-4 inhibitors directly. Here we present the efficacy data from a 24-week study in patients with type 2 diabetes mellitus (T2DM) and severe renal impairment (RI) comparing vildagliptin (VILDA) and sitagliptin (SITA). VILDA is mostly hydrolyzed to inactive metabolites; approximately 20% is excreted as unchanged drug. In patients with severe RI slower elimination effectively doubles the period of time that VILDA prevents GLP-1 and GIP degradation. Therefore, a 50 mg dose once daily provides full efficacy. SITA is essentially excreted unchanged by the kidney. In patients with severe RI, C_{max} increases four-fold, requiring dose reduction. Thus, the expected maximal effective dose and the dose mandated in the label in patients with severe RI is 50 mg qd for VILDA and 25 mg qd for SITA, which have been compared in this study.

Materials and methods: Patients with T2DM (N=148), drug-naïve or on treatment (HbA1c 6.5%-10%), and severe RI [eGFR (MDRD) < 30 ml/min/1.73m²], including patients on haemodialysis (N=12), were randomized in this multicenter, double-blind, parallel-group study to receive VILDA 50 mg qd (N=83) or SITA 25 mg qd (N=65) for 24 weeks in addition to their continued background antidiabetic therapy. Changes in HbA1c and fasting plasma glucose (FPG) from baseline to study endpoint were compared between treatments using an ANCOVA model. Hypoglycaemia was defined as

symptoms suggestive of hypoglycaemia confirmed by a self-monitored blood glucose measurement of <3.1 mmol/l plasma glucose equivalent.

Results: Mean age of the randomized patients was 66.8 years, BMI 33.2 kg/m² and T2DM duration 19.2 years; 80% of the patients were receiving insulin therapy alone or in combination with oral antidiabetic drugs. Mean eGFR was 19.7 and 20.4 ml/min/1.73m² in the VILDA and SITA groups, respectively. At the end of treatment, mean HbA1c was reduced by 0.54% from a lower baseline of 7.52% with VILDA and by 0.56% from a baseline of 7.80% with SITA (p=NS between treatments). Twice as many patients in the VILDA group achieved an HbA1c target ≤6.5% compared with SITA (29.0% vs 14.3%, p=0.050). FPG was reduced by 0.47 mmol/l with VILDA compared to a slight increase of 0.16 mmol/l with SITA (between-group difference: -0.63 mmol/l; p=0.185). While the incidence of hypoglycaemia was similar between treatments (15.7% vs 15.4%), adverse events likely related to hypoglycaemia (hyperhidrosis, tremor, asthenia) as well as asymptomatic low blood glucose were markedly less frequent with VILDA than SITA (7.2% vs 13.8%; 8.4% vs 16.9%; 6.0% vs 21.5%; and 4.9% vs 9.2%, respectively). Overall safety was similar between the two treatments.

Conclusion: In patients with T2DM and severe RI, VILDA and SITA produced similar and clinically relevant HbA1c reductions. However, more patients achieved an HbA1c goal ≤6.5% with VILDA compared to SITA which was not associated with an increase in confirmed (<3.1 mmol/l) hypoglycaemia. The lower levels of glycaemia achieved with VILDA may have been due to the lower rate of symptoms suggestive of hypoglycaemia which typically occur at plasma glucose >3.1 mmol/l, where glucagon counter-regulation is most important. This may be attributed to the previously described effect of VILDA to improve glucagon counter-regulation by maintaining meal-induced increases in GIP during inter-meal periods.

Clinical Trial Registration Number: NCT00616811

Supported by: Novartis

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Pharmacokinetics of once weekly dulaglutide in special populations

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Background and aims: For use in a broad type 2 diabetes mellitus (T2DM) population, dulaglutide pharmacokinetics (PK) were characterized in subjects with renal or hepatic impairment.

Materials and methods: Two separate open-label, single-dose studies assessed dulaglutide 1.5 mg PK in subjects with hepatic (n=15) or renal impairment (n=32), relative to healthy subjects (n=11 and 16, respectively). Both studies included mild, moderate and severe impairment; the renal study also included end stage renal disease.

Results: Dulaglutide exposure (area under concentration-time curve [AUC] and maximum concentration [C_{max}]) was <30% higher in renal impairment groups versus controls. There was no relationship between PK parameters and renal function based on estimated glomerular filtration rate. In addition, there was no statistically significant linear relationship at the 5% significance level between exposure and creatinine clearance (CrCL). A statistically significant linear relationship was observed between CrCL and dulaglutide apparent clearance (CL/F); however the relationship was weak based on its small slope (p=0.0133) and goodness of fit (r²=0.1315). Across all hepatic groups, impaired subjects had lower exposure compared to controls. There was no trend in exposure relative to degree of hepatic impairment, with the largest difference observed in subjects with moderate impairment (C_{max} and AUC values approximately 70% and 67% of controls, respectively). No notable differences in safety profiles were seen between subjects with hepatic or renal impairment and healthy subjects.

Conclusion: There were no clinically relevant effects of renal or hepatic impairment on dulaglutide PK. Dulaglutide can be administered once weekly to patients with renal or hepatic impairment, without dose adjustment.

Clinical Trial Registration Number: NCT01253304

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Liraglutide 3.0 mg improves insulin secretion and action in overweight/obese adults without type 2 diabetes: the SCALE obesity and prediabetes trial

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Background and aims: Obesity is commonly associated with impaired insulin sensitivity and pancreatic beta-cell dysfunction; both implicated in the pathogenesis of type 2 diabetes (T2D). This trial investigated the effects of liraglutide 3.0 mg, as adjunct to diet and exercise, on weight loss (primary endpoint) and insulin secretion and action in overweight/obese individuals with and without prediabetes at screening.

Materials and methods: Adults (BMI ≥27 kg/m² with ≥1 comorbidity or ≥30 kg/m²) were advised on a 500 kcal/day deficit diet and exercise programme and randomised 2:1 to once-daily s.c. liraglutide 3.0 mg or placebo. Randomisation was stratified by prediabetes status (ADA 2010) and BMI at screening. AUCs for glucose, insulin and C-peptide during a 75-g 120 min OGTT at week 0 and 56 were estimated (least square [LS] means, full analysis set [FAS] with LOCF), as were indices of insulin secretion (IS, C-peptide deconvolution method), insulin sensitivity (SI, Matsuda index) and beta-cell function (disposition index [DI], IS*SI) (completers, exploratory analyses).

Results: Baseline characteristics: age 45.1 years, 79% female, weight 106.2 kg, BMI 38.3 kg/m², 61.2% with prediabetes. At week 56, individuals on liraglutide 3.0 mg (n=2437) had weight loss of 8.0% vs 2.6% with placebo (n=1225) (estimated treatment difference [ETD] -5.4%, p<0.0001, LSmeans, FAS with LOCF, ANCOVA). Fasting and post-load glycaemia was improved with liraglutide 3.0 mg, as both FPG and glucose AUC were lower with liraglutide 3.0 mg vs placebo (ETD -0.38 mmol/L and -2.02 h*mmol/L, respectively; p<0.0001, ANCOVA). Post-load insulin and C-peptide were also improved with liraglutide 3.0 mg vs placebo (estimated treatment ratios 1.10 and 1.07, respectively; p<0.0001, ANCOVA on log scale). Improvements applied to individuals both with and without prediabetes, but glucose-lowering was most prominent in individuals with prediabetes (p<0.0001). Improved post-load glucose with liraglutide 3.0 mg vs placebo, was accompanied by increased IS (11% vs 1%), increased SI (21% vs 10%) and improved beta-cell function (35% vs 11%) (all p<0.0001, ANOVA). Similar effects were seen in individuals both with and without prediabetes.

Conclusion: Liraglutide 3.0 mg, as adjunct to diet and exercise, led to weight loss and improvements in insulin secretion and action, all of which likely explain the observed improvements in fasting and post-load glycaemia in overweight and obese individuals with and without prediabetes.

Clinical Trial Registration Number: NCT01272219

Supported by: Novo Nordisk

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Pharmacokinetics and tolerability of a single dose of semaglutide, a once-weekly human GLP-1 analogue, in subjects with and without renal impairment

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Background and aims: The pharmacokinetics and tolerability of semaglutide, a once-weekly, human GLP-1 analogue in development for treatment of type 2 diabetes, were investigated in subjects with and without renal impairment.

Materials and methods: Using the Cockcroft-Gault formula, 56 subjects were assigned to one of five renal function groups and received a single dose of 0.5 mg s. c. semaglutide. In the end-stage renal disease (ESRD) group, semaglutide was given 1-24 h after haemodialysis, with no haemodialysis for 48 h post dose. Semaglutide plasma concentration was assessed regularly up to 480 h (~20 days) post dose; the primary endpoint was area under the semaglutide time-concentration curve from time zero to infinity (AUC_{0-∞}).

Secondary endpoints included other pharmacokinetic parameters (C_{\max} , t_{\max} and $t_{1/2}$), safety and tolerability of semaglutide.

Results: Exposure of semaglutide in subjects with mild or moderate renal impairment or ESRD was similar to that in subjects with normal renal function (Table). Subjects with severe renal impairment had a 22% higher exposure of semaglutide than those with normal renal function and the 95% CI (1.02, 1.47) exceeded the 'no effect' limits (0.70, 1.43). One subject with severe renal impairment reported two major hypoglycaemic events. There were no appreciable changes in laboratory safety parameters or vital signs; no serious adverse events (AEs) were noted. One subject withdrew due to gastrointestinal AEs.

Conclusion: In three of four groups with renal impairment, there was no effect on semaglutide exposure. In the severe renal impairment group, semaglutide exposure was increased. Semaglutide was well tolerated. The long-term effect of semaglutide on renal impairment will be investigated in future trials.

Parameter	Normal renal function (estimated CrCl >80 ml/min) (n=14)	Mild renal impairment (estimated CrCl >50–≤80 ml/min) (n=11)	Moderate renal impairment (estimated CrCl >30–≤50 ml/min) (n=11)	Severe renal impairment (estimated CrCl ≤30 ml/min) (n=10)	End-stage renal disease (requiring dialysis) (n=10)
AUC _{0–∞} , nmol·h/l	2600 (26)	2615 (20) ^b	2999 (19) ^c	3179 (21)	2567 (17) ^b
C _{max} , nmol/l	10 (31)	10 (21)	9 (32)	10 (33)	7 (24) ^b
t _{max} , h	24 (8, 66)	32 (8, 96)	24 (14, 96)	41 (16, 96)	51 (28, 72) ^b
t _{1/2} , h	184 (27)	171 (24) ^b	203 (29) ^c	228 (56)	247 (45) ^b
AUC _{0–∞} estimated ratio (95% CI)	–	1.01 (0.83, 1.22)	1.15 (0.96, 1.38)	1.22 (1.02, 1.47)	0.99 (0.82, 1.19)

CrCl, creatinine clearance; AUC_{0–∞}, area under the curve from time 0 to infinity; C_{max}, maximum concentration; t_{max}, time to maximum concentration; t_{1/2}, elimination half-life.

^a The pharmacokinetic population comprised all subjects exposed to 0.5 mg s.c. semaglutide with an evaluable drug profile.

Values are geometric means (CV (%)) for AUC_{0–∞} and C_{max}; median (min, max) for t_{max}; and mean (SD) for t_{1/2}.

Corresponding parameter could only be estimated for: ^b n=9; ^c n=10.

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Supported by: Novo Nordisk

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VIRTUE study: effects of vildagliptin versus sulphonylureas as monotherapy or combined with metformin in Muslim patients with type 2 diabetes fasting during Ramadan

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Background and aims: For Muslim patients with type 2 diabetes mellitus (T2DM), fasting during Ramadan can increase the risk of serious complications such as hypoglycaemia. This post-hoc analysis of the VIRTUE study assessed, in routine clinical practice, the effectiveness and safety of vildagliptin relative to sulphonylureas (SUs) as monotherapy or when combined with metformin in Muslim patients with T2DM fasting during Ramadan.

Materials and methods: VIRTUE was a multicentre, prospective, 16-week, observational study conducted in the Middle East and Asia, and included adult Muslim patients with T2DM who were receiving vildagliptin or SUs (either as monotherapy or combined with metformin as per routine care) for ≥4 weeks and <3 years prior to the start of fasting. The primary endpoint was the proportion of patients with ≥1 hypoglycaemic event (HE) during Ramadan. Secondary endpoints included change from baseline in HbA_{1c} and body weight, and safety assessments.

Results: Overall, 145 patients received monotherapy (vildagliptin, N=62; SU, N=83) and 1,148 received dual therapy (vildagliptin + metformin, N=607; SU + metformin, N=541). Fewer patients experienced ≥1 HE with vildagliptin

compared with SUs, both as monotherapy and when combined with metformin, the latter achieving statistical significance ($p<0.001$; Table). No grade 2 (severe) HEs were reported with vildagliptin therapy, compared with four patients receiving SU + metformin ($p=0.048$ vs vildagliptin + metformin). Pre- to post-Ramadan changes in HbA_{1c} and body weight were also assessed in vildagliptin and SU treatment groups (Table). Vildagliptin therapy was generally well tolerated. The incidence of adverse events with vildagliptin compared with SUs was 9.7% vs 18.1%, respectively, for monotherapy and 10.2% vs 23.5% for combination with metformin. The most common AEs were hypoglycaemia, pyrexia and nausea.

Conclusion: In this post-hoc analysis, fewer patients experienced HEs with vildagliptin compared with SUs; this achieved significance in patients receiving combination therapy with metformin. In addition, vildagliptin +/- metformin was associated with good glycaemic and weight control, and was well tolerated in this population. As such, vildagliptin may be a useful treatment option for patients with T2DM fasting during Ramadan.

Table. Effects of vildagliptin versus SU monotherapy and in combination with metformin on incidence of HEs, HbA_{1c} and body weight

	Monotherapy		Combination with metformin	
	Vildagliptin	SU	Vildagliptin	SU
Patients with ≥1 HE during fasting (%)	6.5	14.5	5.3*	20.6
Mean change ^{a,b} in HbA _{1c} (%)	-0.45	0.19	-0.22	-0.01
Mean change ^{a,b} in body weight (kg)	-0.31	0.10	-0.81	-0.17

* $p<0.001$ vildagliptin + metformin vs SU + metformin. ^aFrom pre-fasting baseline, ^bbetween-group comparisons not carried out for secondary endpoints

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Enhanced glucose-lowering effect of dipeptidyl peptidase-4 inhibitor in subjects with type 2 diabetes who have a history of gastrectomy

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Background and aims: It has been reported that glucagon like peptide-1 (GLP-1) exhibits its effects not only directly through its receptor on the target cell membrane but also via central nervous system. Hepatic branch vagotomy reportedly decreases insulin secretion in rodents. On the other hand, accelerated gastric excretion in subjects with gastrectomy affects postprandial absorption of nutrients and secretion pattern of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). In this study, we investigated whether the effect of dipeptidyl peptidase-4 (DPP-4) inhibitor is different between the subjects with and without the history of gastrectomy.

Materials and methods: Among patients with type 2 diabetes who were prescribed with DPP-4 inhibitor during 2010 to 2013 at our clinic, those who have the history of gastrectomy more than 6 months before (50±11.4 months) the administration of DPP-4 inhibitor were retrospectively identified from our database (gastrectomy group). Also, patients without the history of gastrectomy who were prescribed with DPP-4 inhibitor (n=20; male: n=13) were randomly selected as a control group. The HbA_{1c} levels measured before and three and six months after DPP-4 inhibitor administration were evaluated. Each value is shown in mean ± SE.

Results: A total of 10 subjects (male: n=8) were identified to meet the conditions (50mg of sitagliptin: n=6, 50mg of vildagliptin: n=4). Control group received 50mg of sitagliptin (n=18), 50mg of vildagliptin (n=1) and 5mg of linagliptin (n=1). The drugs used in combination were sulphonylureas (n=5), insulin (n=2), biganide (n=1), alfa-glucosidase inhibitor (n=1), glinide (n=1) in gastrectomy group, and sulphonylureas (n=11), insulin (n=4), biganides (n=5), and alfa-glucosidase inhibitor (n=1) in control group. Age (71.6±2.5 vs 69.1±2.7 years; gastrectomy group vs control group, $p=0.50$), body mass index (22.5±1.1 vs 24.1±0.7 kg/m²; $p=0.22$) and HbA_{1c} levels before DPP-4 inhibitor administration (8.1±0.1 vs 7.9±0.1 %; $p=0.18$) were not different between two groups. DPP-4 inhibitor administration significantly decreased HbA_{1c} levels in both groups by -1.11±0.20 % in gastrectomy group ($p<0.01$) and -0.88±0.11% in control group ($p<0.01$) at 3 months, and by -1.25±0.11

% ($p<0.01$) and -0.82 ± 0.12 % ($p<0.01$) at 6 months; however, gastrectomy group showed significantly greater reduction compared to control group at 6 months ($p=0.02$).

Conclusion: Our results suggest that glucose-lowering effect of DPP-4 inhibitor is enhanced in the patients who have a history of gastrectomy. Although the study with larger number of subjects will be needed to confirm our results, it is conceivable that enhanced secretion of incretins after gastrectomy might have contributed to the results.

PS 069 Safety of DPP4 inhibitors

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Safety of linagliptin in 8778 patients with type 2 diabetes mellitus: pooled analysis of 23 placebo-controlled randomised clinical trials

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Background and aims: Linagliptin is a DPP-4 inhibitor approved for the treatment of type 2 diabetes mellitus. A robust, global clinical trials program has investigated the efficacy and safety of linagliptin in adult patients. In these trials, linagliptin was given as monotherapy or as add-on to common antidiabetes drugs, such as metformin, sulphonylureas, thiazolidinediones, or insulin. This pooled analysis was undertaken to assess the overall safety of linagliptin in a larger group of patients than would be available in a single randomised trial.

Materials and methods: Tolerability was evaluated by incidence and intensity of adverse events (AEs), AEs of special interest (heart failure, pancreatitis, and pancreatic cancer), and incidence and intensity of hypoglycaemia. Data were analysed for 8778 patients (linagliptin, $n=5488$; placebo, $n=3290$) participating in 23 placebo-controlled randomised trials with durations ≤ 54 weeks. Data were assessed descriptively.

Results: Total exposure was 2883.6 patient years (mean, 192 ± 127 days) and 1957.8 patient years (mean, 217 ± 151 days) in the linagliptin and placebo groups, respectively. Linagliptin was well tolerated, with AEs occurring in a similar proportion of linagliptin and placebo patients (58.0% and 62.3%, respectively). Drug-related AEs were observed in 11.8% and 13.3% of patients, respectively. Heart failure was observed in 0.5% and 0.2% of patients, respectively. Pancreatitis was reported in 0.1% of both groups. One pancreatic carcinoma occurred in the linagliptin group. Investigator-defined hypoglycaemia occurred in 10.6% and 12.2% of linagliptin and placebo patients, respectively (linagliptin, $n=5303$; placebo, $n=3084$). Severe hypoglycaemia was experienced by 0.4% and 0.5% of patients, respectively. Most hypoglycaemia occurred in trials allowing sulphonylureas or insulin as background medications.

Conclusion: These findings provide further evidence for the safety and tolerability of linagliptin in a broad range of patients.

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Lower risk of hypoglycaemia with vildagliptin versus low dose glimepiride in relation to the HbA1c level

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Background and aims: In a previously published study (mean HbA1c 7.3%; $N=3118$), vildagliptin (50 mg bid) showed a reduced risk of hypoglycaemia compared with glimepiride as add-on therapy to metformin after 52 (1.7/16.2%) and 104 (2.3/18.2%) weeks at similar efficacy. Glimepiride (starting dose 2 mg/day) could be titrated to a maximum dose of 6 mg/day. A limitation of the study was the perception that the hypoglycaemia difference was driven by high doses of glimepiride. It was therefore of interest to compare the risk of confirmed hypoglycaemia with vildagliptin to the subgroup of glimepiride patients remaining on the dose of 2 mg/day throughout the study, in addition to all glimepiride patients.

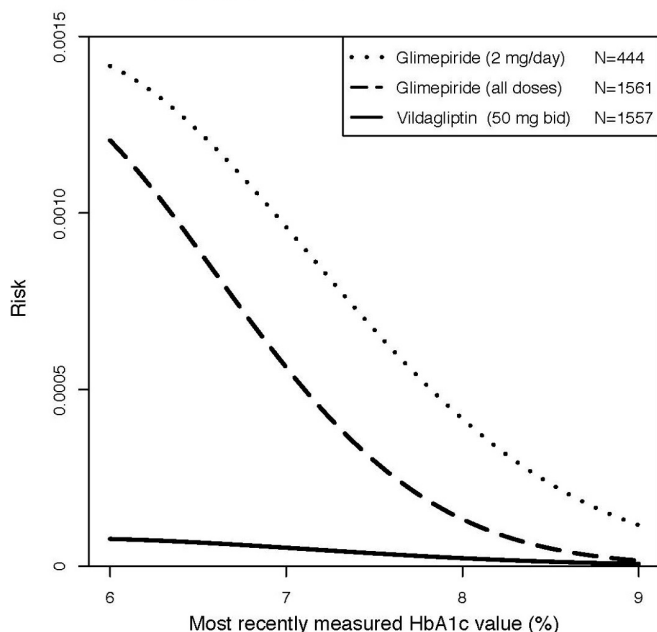
Materials and methods: Comparisons were done by modeling risk as a function of time and last measured HbA1c using discrete event time modelling, with treatment, age and gender as additional covariates. Hypoglycaemia was defined as symptoms suggestive of low blood glucose confirmed by self-monitored blood glucose measurement <3.1 mmol/l plasma glucose equivalent.

Results: The hypoglycaemia risk at week 18 was significantly lower with vildagliptin 50 mg bid compared to glimepiride 2 mg/day, with similar results unadjusted or adjusted for last HbA1c (adjusted HR = 0.05 [95% CI 0.03, 0.10]). The risk of hypoglycaemia was very low with vildagliptin over the full HbA1c

range while the risk with glimepiride 2 mg/day increased with lower HbA1c (Figure). The increase for lower levels of HbA1c was more pronounced in the glimepiride 2 mg/day subgroup than in the full set of patients treated with glimepiride (adjusted HR = 0.08 [95% CI 0.0, 0.15]).

Conclusion: Taken together, the data show a substantially lower risk of confirmed hypoglycaemia with vildagliptin compared to low dose (2 mg/day) glimepiride, and indicate that the previously reported results are not driven by high doses of glimepiride.

Risk of confirmed hypoglycaemia



Supported by: Novartis

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Incidence of fractures in patients with type 2 diabetes in the SAVOR-TIMI 53 trial

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Background and aims: Patients with type 2 diabetes have an increased risk of bone fractures, the predisposing factors for which in diabetes are unknown. In the SAVOR-TIMI 53 trial, fractures were considered an adverse event of special interest (AESI) and information regarding fractures was actively collected by the investigators.

Materials and methods: We compared the incidence of fractures among the 8,280 patients who were assigned to treatment with saxagliptin to the incidence in the 8,212 patients who were assigned to placebo.

Results: During the median follow-up of 2.1 years, 235 (2.8%) and 236 (2.9%) patients in the saxagliptin and placebo groups experienced a fracture; HR (95% CI) = 0.99 (0.83–1.19). The treatment exposure-adjusted rate of bone fracture (first event only) was 14 per 1000 patient-years of follow-up in both groups. Fracture risk was similar in patients treated with saxagliptin or placebo across different subgroups defined by race, cardiovascular risk, renal function and duration of diabetes. A multivariable analysis of the entire study population showed the risk of fracture was associated with older age

($p=0.001$), female gender ($p<0.0001$), longer diabetes duration ($p<0.0001$), elevated HbA1c ($p=0.03$) and treatment with TZDs ($p=0.03$). Fracture risk was not associated with body mass index, estimated glomerular filtration rate, or the use of other anti-diabetic drugs.

Conclusion: The SAVOR-TIMI 53 trial provides important data regarding the risk of fractures in a large population of patients with type 2 diabetes. Treatment with saxagliptin was not associated with an increased risk of fractures. The association between longer diabetes duration and poor glycemic control to increased risk of bone fracture is an intriguing finding.

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Assessment of heart failure risk with vildagliptin in diabetic patients: pooled analysis of safety data from approximately 17,000 patients

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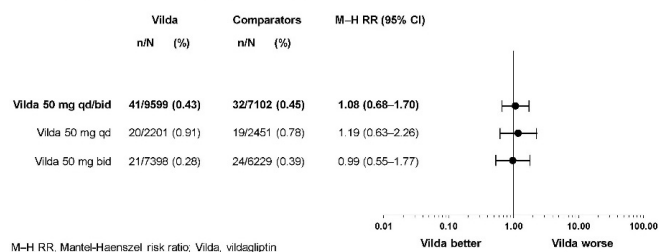
Background and aims: Heart failure (HF) is an important and disabling complication of type 2 diabetes mellitus (T2DM). Two recently completed DPP4 inhibitor cardio-vascular (CV) outcome trials (SAVOR-TIMI-53 and EXAMINE) confirmed the overall CV safety of this class, but also triggered a discussion of a potential HF risk with these agents. We present a meta-analysis of prospectively adjudicated HF events from the vildagliptin pooled database with approximately 17,000 patients.

Materials and methods: Data from 40 randomized, double blind, placebo or active-controlled Phase III vildagliptin studies of ≥ 12 -week duration were pooled. Vildagliptin data on the approved doses of 50 mg qd or bid were considered and analysed consistent with FDA guidance on CV safety. Vildagliptin was used as a monotherapy or in combination with other anti-diabetic drugs in a broad T2DM population including patients with HF NYHA Class I-III. All reported HF events were prospectively adjudicated by independent experts as either 1) HF requiring hospitalization or 2) new onset of HF. The Mantel-Haenszel (M-H) relative risk (RR) of confirmed HF events with vildagliptin vs. comparators (all non-vildagliptin treatments) was calculated.

Results: There were 9599 patients exposed to vildagliptin (9250 subject year exposure [SYE]) compared to 7847 exposed to comparators (7317 SYE). Baseline demographic and clinical characteristics were comparable between treatment groups. Over 40% of patients had 2 or more CV risk factors and approximately 18% had a previous history of cardio-cerebro-vascular disease. Confirmed HF events were reported in 41 (0.43%) patients on vildagliptin 50 mg qd/bid dose and in 32 (0.45%) patients on comparators (Figure) with M-H RR of 1.08 (95% CI 0.68–1.70). No dose response was observed.

Conclusion: This analysis of prospectively adjudicated HF events from the large vildagliptin database indicated that vildagliptin is not associated with increased risk of HF in patients with T2DM relative to comparators.

FIGURE: Incidences and risk ratios for adjudicated heart failure events requiring hospitalization or new onset of heart failure events with vildagliptin vs. comparators



M-H RR, Mantel-Haenszel risk ratio; Vilda, vildagliptin

Supported by: Novartis

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Safety profile of the DPP-4 inhibitors vildagliptin and sitagliptin in patients with type 2 diabetes and severe renal impairment

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Background and aims: Therapeutic management of type 2 diabetes mellitus (T2DM) with declined kidney function is challenging especially in patients with severe renal impairment (RI). Since the actions of the incretin hormones GLP-1 and GIP are glucose-sensitive, hypoglycaemia secondary to increased drug exposure is unlikely to occur with DPP-4 inhibitors (DPP-4i), making this drug class very attractive in treating patients with RI. It has been suggested that safety profiles of DPP-4i may differ in patients with RI, driven by whether or not unchanged drug or metabolites are renally excreted. Here we present a large pooled analysis of safety data for two different DPP-4i eliminated via the kidney, vildagliptin (Vilda), which is primarily hydrolyzed, and sitagliptin (Sita), excreted as unchanged drug, from two studies in 368 patients with T2DM and severe RI.

Materials and methods: Data were pooled across two double-blind studies of similar design (a new study comparing Vilda and Sita at the doses recommended by label in severe RI and a previously reported study comparing Vilda and placebo [Pbo]). Patients with severe RI [eGFR (MDRD) <30 mL/min/1.73m²], including patients on haemodialysis (N=16) were treated with Vilda 50 mg qd (N=206), Pbo (N=97) or Sita 25 mg qd (N=65) for 24 weeks. Absolute incidence rates were calculated for all adverse events (AEs), serious AEs (SAEs), discontinuation due to AEs, and deaths. Laboratory data were also analyzed.

Results: Mean age of the patients was 65.3 years, BMI 31.3 kg/m², HbA1c 7.7% and T2DM duration 18.5 years; 81.3% of patients were treated with insulin as monotherapy or in combination with oral antidiabetics. The incidence of AEs, SAEs, discontinuations due to AEs and deaths were overall comparable between Vilda, Sita and Pbo as shown in Table, which also presents the most commonly reported AEs. The incidence of cardiac events was also similar between treatments (12.6%, 15.4% and 12.4% with Vilda, Sita and Pbo, respectively). Hepatobiliary AEs were infrequent in all 3 groups (1.0%, 1.5% and 0% of patients, respectively) as were persistent elevations ≥3xULN of hepatic enzymes alanine or aspartate aminotransferase (0.5%, 0% and 1.1%, respectively). No cases of pancreatitis were reported in either group. There was no deterioration of renal function with Vilda or Sita compared with Pbo. While a limited number of patients with end stage renal disease on haemodialysis was included in the study (Vilda=8 and Sita=6), the safety data did not indicate that these patients were at increased risk compared to the population with severe RI.

Conclusion: Taken together, in a pooled analysis of patients with severe RI, Vilda and Sita had overall safety profiles comparable with Pbo, indicating that two different DPP-4i that are excreted via the kidney as unchanged drug or metabolites can be safely used in patients with T2DM and advanced RI at the doses recommended by the label.

Table. Adverse events in patients with T2DM and severe renal impairment

	Vildagliptin 50 mg qd N=206 n (%)	Sitagliptin 25 mg qd N=65 n (%)	Placebo N=97 n (%)
AEs	158 (76.7)	56 (86.2)	72 (74.2)
SAEs	43 (20.9)	15 (23.1)	20 (20.6)
Discontinuations due to AEs	17 (8.3)	6 (9.2)	6 (6.2)
Deaths	5 (2.4)	2 (3.1)	4 (4.1)
Most common AEs (by preferred term)*			
Edema peripheral	40 (19.4)	16 (24.6)	18 (18.6)
Hypoglycaemia	32 (15.5)	10 (15.4)	12 (12.4)
Dizziness	25 (12.1)	8 (12.3)	10 (10.3)
Hyperhidrosis	19 (9.2)	9 (13.8)	8 (8.2)
Tremor	13 (6.3)	11 (16.9)	1 (1.0)
Asthenia	12 (5.8)	14 (21.5)	6 (6.2)
Nausea	10 (4.9)	9 (13.8)	6 (6.2)

AE, adverse event; SAE, serious adverse event. *≥10% in any treatment group.

Supported by: Novartis

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Cardiovascular safety of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes: meta-analysis of randomised clinical trials

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Background and aims: In the framework of an EU funded study, the “Safety Evaluation of Adverse Reactions in Diabetes (SAFEGUARD) project”, a meta-analysis of randomized clinical trials (RCTs) to assess the cardiovascular (CV) safety of dipeptidyl peptidase-4 inhibitors (DPP-4i) was performed.

Materials and methods: Trials were identified through searches of MEDLINE, EMBASE and Cochrane databases (January 1966–September 2013) using standard search strategies. Reference lists of selected articles were also checked. Eligible studies included published RCTs that compared DPP-4i against placebo/no treatment, or another non-insulin blood glucose lowering agent in individuals with type 2 diabetes and reported major CV events (CV mortality, sudden death, myocardial infarction, stroke, or heart failure). Two reviewers independently assessed trials for inclusion and extracted data. Effect estimates were pooled using fixed or random effects meta-analysis based on exact bivariate non-linear mixed models. Results are reported as relative risks (RR) with 95% confidence intervals (CI).

Results: The electronic search identified 8,168 citations on all non-insulin blood glucose lowering agents, of which 334 were selected. Out of 140 RCTs on DPP-4i, 70 reported at least one CV event. When DPP-4i were compared with placebo/no treatment, no association was suggested with CV mortality (27 studies, 43,381 participants; RR=0.85; 0.71–1.02), sudden death (15 studies, 23,494 participants; RR=1.17; 0.90–1.51), myocardial infarction (20 studies, 27,553 participants; RR=0.95; 0.84–1.09), and ischemic stroke (15 studies, 27,224 participants; RR=1.04; 0.84–1.29). There was an increase in the risk of heart failure in patients treated with DPP-4i compared to placebo (9 studies, 20,349 participants; RR=1.24, 1.03–1.49). For active comparator studies no association was found for CV mortality (24 studies, 22,064 participants; RR=0.92; 0.50–1.70), sudden death (8 studies, 7,130 participants; RR=0.83; 0.23–3.00), myocardial infarction (12 studies, 5,453 participants; RR=0.70; 0.34–1.43), or heart failure (6 studies, 6,180 participants; RR=1.31; 0.50–3.43). DPP-4i reduced the risk of ischemic stroke when compared to active comparator (8 studies, 7,777 participants; RR=0.33; 0.14–0.79).

Conclusion: These results, based on publicly available data, suggest that DPP-4i did not influence the risk of CV mortality or other CV events in comparison with placebo/no treatment, except for an increased risk of heart failure. However, the results are driven by the findings from the “Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR)-Thrombolysis in Myocardial Infarction (TIMI) 53” study and the finding needs to be confirmed by pooling these data with those of other ongoing studies specifically designed to assess the effect on CV end-points. Furthermore, DPP-4i showed a lower risk of ischemic stroke than active comparators. Additional trial data are required before definitively draw conclusions on CV safety of DPP-4i.

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Cardiovascular safety of vildagliptin: an adjudicated meta-analysis of 40 studies

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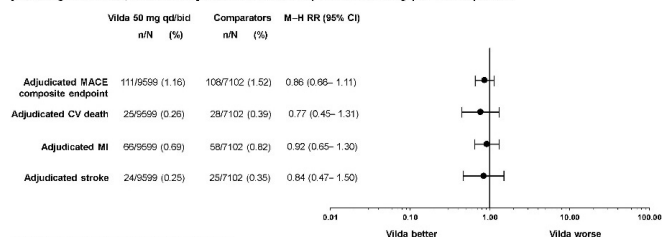
Background and aims: Since the previous assessment of the cardiovascular (CV) safety profile of the DPP-4 inhibitor vildagliptin, a considerable number of additional studies have completed, including trials in high risk patients, such as with congestive heart failure or moderate/several renal impairment. It was therefore important to re-assess the CV safety of vildagliptin in this enlarged pool of studies.

Materials and methods: Patient-level data were pooled from 40 double-blind, controlled studies of vildagliptin, used either as monotherapy or combination therapy, ranging in duration from 12 to ≥ 104 weeks. A retrospective meta-analysis of prospectively adjudicated CV events by a blinded independent expert committee was performed using Mantel-Haenszel risk ratios (RR) to compare the vildagliptin treatment group (approved doses of 50 mg bid and qd) to the comparators groups (placebo and active comparators). The primary endpoint was a major adverse CV events (MACE) composite of myocardial infarction, stroke and CV death. Additional assessments included the individual components of the composite endpoint.

Results: Patients included in the pooled dataset [treated with vildagliptin (N=9599; 9250 subject year exposure [SYE]) or comparators (N=7847; 7317 SYE)] had a mean age of ~ 57 years, slight male predominance ($\sim 55/45\%$), mean BMI of 30.5 kg/m^2 (nearly 50% obese), mean HbA1c of 8.1%, and a mean T2DM duration of 5.6 years; 46% of patients had dyslipidemia, 58% hypertension and 27% were 65 years or older. The incidences and risk ratios for the MACE composite endpoint as well as the individual components are shown in the Figure below. A MACE occurred in 111 (1.16%) patients receiving vildagliptin and 108 (1.52%) patients receiving comparators, the resulting RR being 0.86 (95% CI 0.66, 1.11), indicating no increased risk of MACE with vildagliptin. Similar risk ratios were also seen for each individual component of the composite endpoint (Figure).

Conclusion: This large meta-analysis demonstrates that vildagliptin is not associated with an increased risk for adjudicated MACE events relative to comparators, confirming the CV safety profile of the drug in a largely expanded pooled population.

FIGURE: Incidences and risk ratios for adjudicated major adverse CV (MACE) composite endpoint (consisting of CV death, MI and stroke) and its individual components with vildagliptin vs. comparators



M-H RR, Mantel-Haenszel risk ratio; Vilda, vildagliptin

Supported by: Novartis

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Lower respiratory tract infections and the use of dipeptidyl peptidase-4 inhibitors: a population-based matched cohort study

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Background and aims: It is hypothesized that dipeptidyl peptidase-4 (DPP-4) inhibitors, also known as gliptins, may alter the immune response and play a role in the occurrence of infections. The aim of this study was to determine whether the use of DPP-4 inhibitors is associated with an increased risk of community-acquired lower respiratory tract infection, when compared to sulfonylureas.

Materials and methods: A population-based matched cohort study was conducted among linkable patients with records in both the United Kingdom Clinical Practice Research Datalink (CPRD) and the Hospital Episodes Statistics (HES) database. Between January 1, 2007 and March 31, 2012, each new user of DPP-4 inhibitors was matched to two patients prescribed sulfonylureas either in monotherapy or in combination, on three variables: age, duration of treated diabetes (defined as the time between a first ever non-insulin prescription and cohort entry) and use of sulfonylurea in the prior year. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of community-acquired lower respiratory tract infection, comparing DPP-4 inhibitors users to sulfonylurea users. In secondary analyses, types of DPP-4 inhibitors, and the outcome of hospitalization for community-acquired pneumonia were assessed. All models were adjusted for comorbidities and diabetes-related variables (such as hemoglobin A1c and previous use of anti-diabetic drugs).

Results: Overall, 6933 new users of DPP-4 inhibitors were compared to 17,026 users of sulfonylureas. The crude incidence rates of community-acquired lower respiratory tract infection was 8.44 per 100 person-years (95% CI: 7.71 - 9.23) for DPP-4 inhibitors users and 7.54 (95% CI: 7.21 - 7.89) for

sulfonylurea users. Compared to sulfonylureas, the use of DPP-4 inhibitors was not associated with an increased risk of community-acquired lower respiratory tract infection and hospitalization for community-acquired pneumonia (0.90, 95% CI: 0.78 - 1.04 and 0.76, 95% CI: 0.44 - 1.31, respectively) (table 1). In the subgroup analysis by type of DPP-4 inhibitors, the risk of community-acquired lower respiratory tract infection ranged from a 17% decreased risk (HR: 0.83, 95% CI: 0.71 - 0.97) for sitagliptin, to a 31% increased risk for vildagliptin (HR: 1.31, 95% CI: 1.00 - 1.72).

Conclusion: Overall, the use of DPP-4 inhibitors as a class does not appear to be associated with an increased risk of community-acquired lower respiratory tract infections or hospitalization for community-acquired pneumonia. Additional research is needed to determine whether there is a within-class effect.

Table 1. Crude and adjusted hazard ratios of community-acquired lower respiratory tract infections associated with the use of DPP-4 inhibitors compared to sulfonylureas*

Exposure group	No.	Events	Person-years	Incidence rate per 1000 person-years (95% CI)	Crude HR (95% CI)	Adjusted [†] HR (95% CI)
Sulfonylureas	13,866	1514	20,846	72.63 (69.06–76.38)	1.00 (Reference)	1.00 (Reference)
DPP-4 inhibitors	6933	471	5,582	84.37 (77.09–92.35)	1.03 (0.93–1.15)	0.90 (0.78–1.04)

HR, hazard ratio; CI, confidence interval

*Patients were matched on age, duration of treated diabetes (defined as the time between a first ever non-insulin prescription and cohort entry) and use of sulfonylurea in the prior year.

[†]Adjusted for age, sex, body mass index, HbA1c categories, smoking status, year of cohort entry, duration of treated diabetes, alcohol use, prior use of insulin, metformin, sulfonylurea, thiazolidinediones and other anti-diabetic drug, number of anti-diabetic drug ever used, lower respiratory tract infection, asthma, chronic obstructive pulmonary disease, bronchiectasis, >4 physician visits in the year prior to cohort entry, use of immunosuppressive agents, inhaled bronchodilators, inhaled corticosteroids, non-topical antibiotics, non-topical corticosteroids, use of influenza or pneumococcal vaccines.

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Pregnancy outcomes after unintentional exposure to vildagliptin

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Background and aims: Type 2 diabetes mellitus (T2DM) is increasingly diagnosed in younger adults and as the population of women with diabetes becomes younger, T2DM and pregnancy increasingly co-exist. Pregnancies complicated by diabetes are associated with an increased risk of adverse outcomes such as preterm delivery, abortions, congenital abnormalities and neonatal mortality. However, data on outcomes in women exposed to newer oral anti-diabetes drugs (OADs), including vildagliptin, are limited. In the absence of adequate studies, most OADs carry warnings against use during pregnancy. Nevertheless, some use of these drugs occurs during pregnancy, either accidentally or intentionally. This analysis was performed to evaluate outcomes in pregnant women exposed to vildagliptin treatment.

Materials and methods: The Novartis Safety Database was searched to identify all known records (cut-off date: 30 September 2013) of exposure to vildagliptin (\pm metformin) during pregnancy. The database includes clinical study and post-marketing surveillance reports, as well as spontaneous reports. Maternal outcomes (number of live births, pre-term deliveries, abortions or caesarean sections) and neonatal outcomes (number of congenital anomalies or deaths) were analysed and presented as descriptive data when available.

Results: A total of 32 pregnancies in 31 women with T2DM were identified from the database: 14 cases from controlled clinical trials, 15 spontaneous reports and 3 cases from post-marketing studies. The availability of demographic and baseline characteristics data was limited. The mean age was 33.9 (range 19 - 44) years and 29.0% of women had a previous history of an abortion (elective or spontaneous). Limited information was available on cumulative vildagliptin dose or trimester(s) of exposure. 23 out of 32 pregnancies (71.9%) resulted in a live birth and more than half of all the pregnancies (n=18, 56.3%) had an unremarkable outcome of normal newborns without complications. Three (9.4%) pregnancies resulted in normal newborns with pregnancy complications (one case of gestational hypertension, haemorrhage during pregnancy and placenta previa) and two (6.3%) resulted in preterm deliveries. Four caesarean sections were reported (all live births), one in the preterm and three in term pregnancies. There were eight (24.9%) spontaneous or missed abortions, but their relationship to vildagliptin was unclear. Additionally one case of therapeutic abortion was reported. No congenital abnormalities or neonatal deaths were reported.

Conclusion: Although the numbers are low, this analysis provides preliminary information on pregnancy outcomes in T2DM after exposure to vildagliptin. No prospective studies of vildagliptin have been performed in pregnant women with T2DM. As with most OADs, vildagliptin is also not recommended for use during pregnancy.

Supported by: Novartis Pharma AG

PS 070 Clinical studies with DPP4 inhibitors

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Oral glucose lowering with linagliptin plus metformin is a viable initial treatment strategy in patients with newly diagnosed type 2 diabetes and marked hyperglycaemia

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Background and aims: Newly diagnosed type 2 diabetes (T2D) patients commonly present with marked hyperglycaemia. This condition has rarely been studied for novel oral diabetes drugs and insulin is often proposed as the preferred starting therapy.

Materials and methods: We explored oral glucose-lowering combination therapy in newly diagnosed (≤ 12 months) T2D patients with marked hyperglycaemia ($n=316$) utilising prespecified exploratory subgroup analyses from a randomised double-blind study of initial combination of linagliptin+metformin versus linagliptin. Baseline mean \pm SD age and HbA1c was 48.8 ± 11.0 years and $9.8\pm 1.1\%$, respectively. The primary endpoint was HbA1c change from baseline to week 24.

Results: Mean \pm SE HbA1c reduction was $-3.4\pm 0.2\%$ versus $-2.5\pm 0.2\%$ with linagliptin+metformin and linagliptin, respectively, in patients with baseline HbA1c $\geq 9.5\%$, and $-2.1\pm 0.2\%$ versus $-1.4\pm 0.2\%$ in patients with baseline HbA1c $< 9.5\%$. Similar HbA1c reductions occurred in all subgroups of age, body-mass index (BMI), renal function, race, and ethnicity (table). Hypoglycaemia was rare (1.9% and 3.2% of patients, respectively) with no severe episodes.

Conclusion: In our analysis of newly diagnosed T2D patients presenting with marked hyperglycaemia, initial linagliptin+metformin elicited consistent HbA1c reductions across different subgroups. Oral glucose-lowering combination therapy may be a viable initial alternative to insulin for effective treatment of these patients.

	Linagliptin 5 mg qd + metformin bid [†]		Linagliptin 5 mg qd		Treatment difference (linagliptin + metformin minus linagliptin)	
	n	Mean \pm SE	n	Mean \pm SE	Mean \pm SE	95% CI
Baseline HbA1c						
<9.5%	56	-2.1 \pm 0.2	52	-1.4 \pm 0.2	-0.7 \pm 0.3	-1.2, -0.2
$\geq 9.5\%$	76	-3.4 \pm 0.2	61	-2.5 \pm 0.2	-0.8 \pm 0.3	-1.3, -0.4
Age						
<35 years	12	-2.5 \pm 0.4	13	-2.2 \pm 0.4	-0.2 \pm 0.5	-1.3, 0.8
35–<50 years	47	-3.0 \pm 0.2	35	-2.3 \pm 0.2	-0.7 \pm 0.3	-1.3, -0.1
50–<65 years	66	-2.7 \pm 0.2	58	-1.9 \pm 0.2	-0.8 \pm 0.2	-1.2, -0.3
≥ 65 years	7	-3.5 \pm 0.5	7	-1.1 \pm 0.5	-2.4 \pm 0.7	-3.8, -1.0
Renal function [‡]						
Normal	102	-2.7 \pm 0.1	89	-2.0 \pm 0.1	-0.7 \pm 0.2	-1.1, -0.3
Impaired	30	-3.1 \pm 0.2	24	-2.0 \pm 0.3	-1.0 \pm 0.4	-1.8, -0.3
BMI, kg/m ²						
<25	28	-3.0 \pm 0.3	18	-2.0 \pm 0.3	-1.0 \pm 0.4	-1.8, -0.2
25–<30	46	-2.9 \pm 0.2	40	-1.9 \pm 0.2	-1.0 \pm 0.3	-1.6, -0.4
30–<35	31	-2.7 \pm 0.2	34	-2.0 \pm 0.2	-0.6 \pm 0.3	-1.3, 0.1
≥ 35	27	-2.7 \pm 0.3	21	-2.2 \pm 0.3	-0.4 \pm 0.4	-1.2, 0.4
Race						
White	83	-2.7 \pm 0.2	65	-2.1 \pm 0.2	-0.6 \pm 0.2	-1.1, -0.2
Black	3	-2.3 \pm 0.8	4	-1.2 \pm 0.7	-1.1 \pm 1.0	-3.1, 1.0
Asian	46	-3.0 \pm 0.2	44	-2.0 \pm 0.2	-1.1 \pm 0.3	-1.6, -0.5
Ethnicity						
Not Hispanic/Latino	104	-2.8 \pm 0.1	89	-1.8 \pm 0.1	-1.0 \pm 0.2	-1.4, -0.6
Hispanic/Latino	28	-3.0 \pm 0.3	24	-3.0 \pm 0.3	-0.1 \pm 0.4	-0.8, 0.7

*ANCOVA model with terms for treatment, continuous baseline HbA1c, subgroup, subgroup by treatment interaction; for analyses of baseline HbA1c subgroups, the term for continuous baseline HbA1c was replaced by the categorical baseline HbA1c.

**Randomised patients who received ≥ 1 dose of study drug and a baseline HbA1c measurement, with no important protocol violations, who completed 24 weeks of treatment without glycaemic rescue, and had an HbA1c measurement at week 24.

[†]Metformin was uptitrated in the first 6 weeks to a maximal dose of 2000 mg/day.

[‡]Estimated creatinine clearance by the Cockcroft-Gault equation: normal renal function is ≥ 90 mL/min; impaired renal function is < 90 mL/min.

Clinical Trial Registration Number: NCT01512979

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Vildagliptin versus insulin as add-on therapy to glimepiride in type 2 diabetes mellitus: results from a randomised controlled trial

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Background and aims: The study was designed to compare the effects of the dipeptidyl-peptidase 4 (DPP-4) inhibitor vildagliptin and insulin, in addition to glimepiride, in patients with type 2 diabetes mellitus (T2DM) who do cannot receive metformin.

Materials and methods: This was a multicenter, randomized, parallel-group controlled study to evaluate the occurrence of hypoglycemic events (HE) and treatment success on treatment with vildagliptin vs. NPH insulin, each in addition to background therapy with glimepiride. T2DM patients with intolerance or contraindications to metformin therapy, and who did not have adequate glycemic control on their previous sulfonylurea monotherapy, were eligible for inclusion. Vildagliptin 50 mg once daily was compared in an open-label design with NPH insulin once daily, in an individually titrated dose, over 24 weeks. Primary efficacy endpoints were a combination of HbA1c target $< 7.0\%$ without HE (< 3.9 mmol/L) and bodyweight gain ($> 3\%$), and rate of confirmed HEs. Secondary efficacy variables, amongst others, included assessment of the Treatment Satisfaction Questionnaire for Medication (TSQM-9).

Results: 162 patients with T2DM and a mean age of 67.7 (SD 10.8) years were enrolled at 47 centers in Germany (83 patients in the vildagliptin group vs. 79 patients in the insulin group). Mean HbA1c at baseline was 7.6% (SD 0.5; $7.61 \pm 0.47\%$ in the vildagliptin group, $7.67 \pm 0.52\%$ in the insulin group; $p=0.510$). After 24 weeks, 13.4% in the vildagliptin group and 29.1% in the insulin group had confirmed HEs, while 11.0% of patients in the vildagliptin group and 23.8% in the insulin group experienced a bodyweight gain $> 3\%$. 48.8% patients in the vildagliptin group and 60.8% patients in the insulin group achieved the HbA1c target level of $< 7.0\%$ at Week 24. Overall, the rate of patients fulfilling the combined criteria was similar in the two treatment groups: 35.4% in the vildagliptin group vs. 34.2% in the NPH insulin group achieved an HbA1c level of $< 7.0\%$ without HEs and bodyweight gain $> 3\%$ (OR=0.985, 95% CI for OR: [0.507; 1.915], $p=0.9646$). Patients' satisfaction

with medication, assessed as TSQM-9 scores for “convenience”, showed an increase from baseline to week 24 for the vildagliptin group and a decrease in the insulin group (9.6 ± 19.1 score points vs. -5.6 ± 22.0 score points; $p < 0.001$). Scores for “effectiveness” and “global satisfaction” increased similarly for both treatment groups.

Conclusion: In the treatment of T2DM in patients with insufficient glycaemic control on sulfonylurea monotherapy, addition of vildagliptin or insulin resulted in a similar number of patients reaching target HbA1c without HEs or bodyweight change $>3\%$. That more patients achieved the HbA1c target of $<7.0\%$ with insulin treatment was expected because of individualized dose titration, but was associated with a doubling of confirmed HEs in this group. Vildagliptin was associated with greater convenience and treatment satisfaction scores compared with insulin. In these patients, the addition of vildagliptin to glimepiride could be considered as a treatment option prior to escalation to insulin treatment, with the advantages of a lower HE rate and greater patient convenience.

Clinical Trial Registration Number: NCT01649466

Supported by: Novartis Pharma GmbH, Germany

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Efficacy of vildagliptin and sitagliptin in reducing fasting plasma glucose in type 2 diabetes mellitus: results from a randomised controlled trial

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Background and aims: This study was designed to compare the effects of dipetidyl-peptidase 4 (DPP-4) inhibitors vildagliptin and sitagliptin, in addition to metformin, on fasting plasma glucose (FPG) levels in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: This study applied a randomized cross-over, open-label, active-controlled design to assess the FPG-lowering abilities of a single pill combination (SPC) of vildagliptin and metformin (50/1000 mg bid) compared to sitagliptin/metformin SPC (50/1000 mg bid). Patients with type 2 diabetes mellitus (T2DM) insufficiently controlled by stable metformin therapy (1000–2000 mg/day) were included. FPG was assessed twice, after 2 weeks' treatment with either medication: on day 14, and again on day 15 after a missed dose the previous evening.

Results: 99 patients with T2DM (35.4% female) and a mean age of 61.2 years (SD 10.1) were enrolled at 15 centers in Germany. Mean HbA1c on screening was 7.5 % (SD 0.68), and mean FPG was 164.3 mg/dL (SD 30.9). After 14 days of treatment, mean FPG was 137.8 mg/dL (SD 28.5) on vildagliptin and 140.1 mg/dL (SD 26.5) on sitagliptin ($p < 0.05$, Wilcoxon). Change of FPG from baseline to day 14 was significantly greater under vildagliptin [-21.9 mg/dL (SD 27.0)] as compared to sitagliptin [-14.5 mg/dL (SD 23.0)] ($p = 0.0196$, Wilcoxon). After a missed evening dose, FPG increased slightly at day 15 in both treatment groups, with a greater increase on vildagliptin than sitagliptin treatment [day 15 FPG: 147.6 mg/dL (SD 29.2) vs. 143.4 mg/dL (SD 27.9), respectively; $p = 0.0473$, Wilcoxon]. Adverse events were infrequent, and were mainly of mild or moderate intensity in both treatment groups.

Conclusion: Both DPP-4 inhibitors, given as SPC with metformin bid, lowered FPG after 14 days of treatment. Vildagliptin produced a significantly greater reduction in FPG versus baseline compared with sitagliptin, and this may have clinical relevance.

Clinical Trial Registration Number: NCT01398592

Supported by: Novartis Pharma GmbH, Germany

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Improvement in postprandial glucose after 2-year treatment with alogliptin in patients with type 2 diabetes inadequately controlled with metformin

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Background and aims: Increased postprandial glucose (PPG) contributes to hyperglycemia in patients with type 2 diabetes (T2D), and may be associated with an increased risk of cardiovascular disease. Alogliptin (ALO) is an oral anti-diabetic agent that improves glycaemic control by enhancing the incretin system in patients with T2D. Based on its mechanism of action, ALO may have a significant impact on PPG. The impact on PPG by ALO has only

been demonstrated in a short-term (16-week) study. In a sub-population of a large 2-year, multicenter, double-blind, randomized clinical trial examining the durability of glycaemic control of ALO compared to glipizide (GLIP), the long-term reduction in PPG by ALO was examined.

Materials and methods: A total of 2639 patients with T2D inadequately controlled by metformin alone were randomized to ALO 12.5mg (A12.5), ALO 25mg (A25) or GLIP (5mg titrating to 20mg) treatment groups. The primary endpoint was HbA1c reduction from baseline (BL) at 52 and 104 weeks. In study centers capable of measuring PPG, 2-hour PPG was measured after a standardized meal at BL, week 12, 26, 52, and 104 visits.

Results: A total of 1418 subjects had a BL PPG measurement. Mean 2-hour PPG levels were similar among treatment groups at BL: 207.5 mg/dL, 203.2 mg/dL, and 197.3 mg/dL in the A12.5 (n=470), A25 (n=492), and glipizide (n=456) groups, respectively. Reduction of PPG occurred in all 3 groups at week 12 and lasted through week 104. At week 104, the LS mean reductions from BL in 2-hour PPG levels were significantly greater with A12.5 (n=230, -12.9 mg/dL; $p=0.014$) and A25 (n=257, -11.4 mg/dL; $p=0.027$) when compared with GLIP (n=214, 0.9 mg/dL). The 2-hour PPG excursion was determined by the 120-min glucose value minus the 0-min glucose value at that visit. At week 104, the LS mean changes from BL in PPG excursions were 6.4 and -6.8 mg/dL in the A12.5 and A25 treatment groups, respectively, vs 2.8 mg/dL in the GLIP treatment group; no statistically significant treatment-group differences were observed.

Conclusion: In this study, ALO reduced PPG over the 2-year treatment period, and this reduction appears to be more sustainable with ALO than that with GLIP.

Clinical Trial Registration Number: NCT00856284

Supported by: Takeda Development Center Americas, Inc.

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Assessing time to insulin use among type 2 diabetes patients treated with sitagliptin or sulfonylurea plus metformin dual therapy

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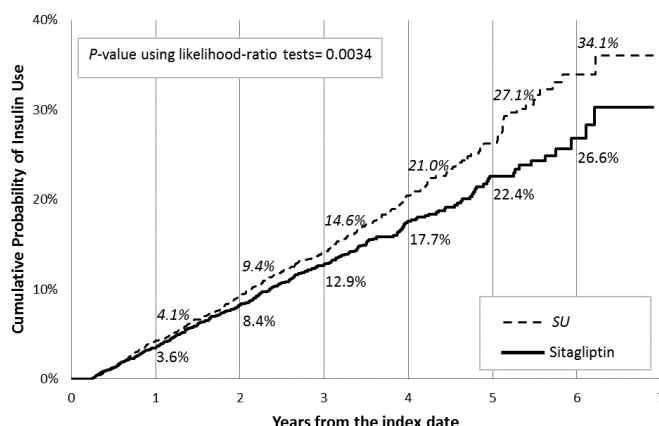
Background and aims: In type 2 diabetes mellitus (T2DM), decline of β -cell function over time leads to the need for treatment intensification and eventually initiation of insulin therapy in many patients (pts). Sitagliptin (SITA) and sulfonylureas (SU) are commonly prescribed after metformin as dual therapy in T2DM. This study assessed the time to insulin initiation among pts treated with MET+SITA in comparison to those treated with MET+SU.

Materials and methods: This retrospective cohort study used a sample from the GE Centricity database. Included were pts with T2DM, ≥ 18 years (yrs), with continuous medical records. Index was the date of the 1st prescription of SITA or SU used as dual therapy with metformin for ≥ 90 days in 2006–13. SITA and SU users were matched 1:1 using propensity score (PSM). Differences in time to insulin use (from index date) between SITA and SU users were assessed using Kaplan-Meier (KM) curves and Cox regression. Conditional logistic regression (CLR) examined the likelihood of insulin use in each of yrs 1–5 post index. Adjustments were made for baseline characteristics. Subgroup analyses for baseline A1C $< 9\%$ or $\geq 9\%$ were then conducted.

Results: PSM produced 3,862 matched pairs. The percent of pts progressing to insulin by yrs 1–6 were 3.6, 8.4, 12.9, 17.7, 22.4, 26.6 for SITA and 4.1, 9.4, 14.6, 21.0, 27.1, 34.1 for SU users, respectively. KM curves for SITA and SU users were significantly different ($p=0.0034$) indicating that SITA users progressed more slowly to insulin initiation than SU users. This remained significant after adjusting for baseline characteristics (HR: 0.76, 95% CI: [0.464, 0.897]). CLR analyses confirmed the robustness of the results (ORs: 0.77; 0.79; 0.81; 0.57; 0.29; for yrs 1–5 respectively, $p < .05$ for Yr 4 and Yr 5). The SITA vs. SU comparison in pts with baseline A1C $< 9\%$ and $\geq 9\%$ produced hazard ratios of: 0.77, 95% CI: [0.621, 0.945], and 0.75, 95% CI: [0.490, 1.145], respectively.

Conclusion: In this matched cohort study, pts with T2DM who started with MET+SITA dual therapy progressed to insulin therapy at a slower rate than those who started with MET+SU dual therapy.

Kaplan Meier Cumulative Distribution Function for Time-to-first-insulin



Supported by: MSD

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Combination with linagliptin improves the tolerability of metformin in type 2 diabetic patients previously labelled as intolerant to metformin

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Background and aims: Metformin is the first-line drug for the treatment of type 2 diabetes but gastrointestinal intolerance to it is frequent. We sought to determine if patients withdrawn from metformin treatment due to intolerance would be able to tolerate it when rechallenged, and if the addition of linagliptin would improve the probability of tolerating metformin.

Materials and methods: From the clinical records of our Diabetes Clinic, we identified type 2 diabetic patients aged 30 to 80 with HbA1c > 7% who had withdrawn from metformin due to gastrointestinal intolerance but had presented no serious adverse effects and had no significant renal insufficiency or other contraindications for metformin use, were not taking incretin-based therapy and were possible candidates for treatment with metformin and/or linagliptin. After at least 6 months from withdrawal, they were randomised to treatment with metformin alone (500 mg twice daily for 2 weeks, then 1000 mg twice daily for 10 additional weeks if the previous dose was tolerated), or with metformin (same pattern) plus linagliptin 5 mg daily, in an open fashion. Tolerance was assessed at weeks 2 and 12 by questionnaire performed by personnel blinded to the treatment status. Fasting glucose and HbA1c were measured at baseline and 12 weeks.

Results: We found 84 consecutive patients who were offered to participate. 25 declined, 30 received metformin plus linagliptin (ML) and 29 metformin alone (M). Their age was 63.2 ± 7.6 years, 56% were female; at baseline their fasting plasma glucose (FPG) was 9.2 ± 1.6 mmol/l and their HbA1c was $8.2 \pm 0.9\%$. There were no significant differences between the groups for any of these variables. In the M group, 8 patients (28%) tolerated the low-dose and 5 (17%) the full-dose metformin (odds/ratio 3.94; 95% CI 1.32–11.76, $p=0.014$). In the ML group, 18 patients (60%) tolerated the low-dose and 14 (47%) the full-dose metformin (odds/ratio 4.20; 95% CI 1.26–13.96, $p=0.019$). By intention to treat, at week 12 the FPG in the M group was reduced by 1.1 ± 0.5 mmol/l and HbA1c was reduced by $0.2 \pm 0.1\%$. In the ML group FPG was reduced by 2.7 ± 1.2 mmol/l and HbA1c was reduced by $0.9 \pm 0.3\%$ (both $p < 0.001$ between groups, non-paired t-test). By protocol (patients that tolerated at least low-dose metformin), at week 12 the FPG in the M group was reduced by 2.5 ± 0.9 mmol/l and HbA1c was reduced by $0.5 \pm 0.2\%$. In the ML group FPG was reduced by 3.2 ± 1.3 mmol/l ($p=0.020$) and HbA1c was reduced by $1.1 \pm 0.4\%$ ($p < 0.001$). Apart from minor gastrointestinal disturbances, the patients did not report any side effects that could be attributed to the studied drugs.

Conclusion: A significant proportion of patients labelled as intolerant to metformin could be successfully rechallenged with it, although only a fraction of them tolerated the full-dose treatment. The addition of linagliptin improved very significantly the probability of a successful rechallenge with metformin, resulting in a clear improvement in glycemic control, without additional tolerability issues.

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Real life effectiveness and safety of vildagliptin compared with other OADs in European type 2 diabetes mellitus patients: results from the EDGE study

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Background and aims: Stepwise treatment intensification is necessary in type 2 diabetes mellitus (T2DM) due to progressive loss of β -cell function. European societies (ESC/EASD) provide algorithms for improving glycaemic and risk factor control with a holistic approach in the management of T2DM and cardiovascular disease; HbA1c goal of $\leq 7\%$, avoiding hypoglycaemia and individualising therapy and glycaemic targets. The purpose of the observational EDGE study was to prospectively evaluate the effectiveness and safety of vildagliptin vs other oral antidiabetes drugs (OADs) in T2DM patients inadequately controlled with monotherapy. Here, we present the post-hoc analysis results for the 12 participating European countries.

Materials and methods: Patients receiving physician-prescribed add-on OAD treatment were assigned to: (i) vildagliptin or (ii) other OADs (except other incretin-based therapies). The primary composite endpoint was the proportion of patients with HbA1c drop $> 0.3\%$, without peripheral oedema, hypoglycaemia, discontinuation due to a gastrointestinal event or weight gain $\geq 5\%$ at 1 year. A secondary composite endpoint was attainment of HbA1c $< 7.0\%$ at 1 year without hypoglycaemia or weight gain $\geq 3\%$.

Results: In Europe, 23038 patients receiving mostly metformin monotherapy were enrolled (total intention to treat: 22073; vildagliptin: 15582; other OADs: 6491). The mean baseline HbA1c was $7.9 \pm 1.3\%$, mean T2DM duration was 6.3 ± 5.6 years and 52.0% were male. Prevalence of risk factors such as hypertension (68.5%) and lipid disorders (50.0%) was high. Micro- and macrovascular complications were present in 9.6% and 18.4% of patients. Reflecting real life setting, there was heterogeneity in patient characteristics among different countries and imbalances between treatment cohorts. Patients in the vildagliptin cohort were younger at baseline (61.8 ± 11.0 years vs 63.4 ± 10.5 years) with slightly higher BMI (30.5 ± 5.3 vs 29.9 ± 4.9) while the cohorts had comparable mean baseline HbA1c ($7.9 \pm 1.3\%$ vs $7.8 \pm 1.2\%$). Both the primary (49.3% vs 46.5%; OR 1.11, 95% CI: 1.05; 1.18; $p < 0.001$) and secondary (34.6% vs 28.5%; OR 1.32, 95% CI: 1.23; 1.42; $p < 0.001$) composite endpoints were reached more often in patients receiving vildagliptin than other OADs. Between-country variability was substantial for both endpoints. The percentage of patients reporting adverse events was low and similar, 5.8% in each cohort. Also hypoglycaemia rates were low, 0.2% for vildagliptin vs 0.8% for other OADs.

Conclusion: In addition to confirming the real world effectiveness and safety of vildagliptin, EDGE highlights the sustained need for improvement in adherence to Europe-specific guidelines (ESC/EASD) for optimised management of glycaemic targets.

Supported by: Novartis

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Duration of maintenance of dual therapy with metformin and sitagliptin in type 2 diabetes: the Odyssée observational studyP. Valensi¹, G. De Pouvourville², N. Benard³, C. Chanut-Vogel³, C. Kempf⁴, C. Moisan³, J. Dallongeville⁵;¹Department of Endocrinology Diabetology Nutrition-Jean Verdier Hospital, CRNH-IdF, CINFO, Bondy, ²ESSEC, Paris,³MSD, Courbevoie, ⁴CSD, PARIS, ⁵Institut Pasteur, Lille, France.

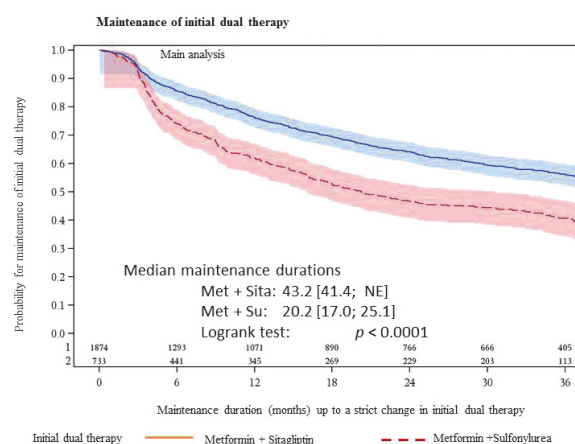
Background and aims: Sulfonylurea and DPP-4 inhibitors are usually prescribed for T2DM patients in combination with metformin. Odyssée, a prospective, real-world, observational study conducted in France in primary care practices, compared the duration of maintenance of treatment without modification (withdrawal, substitution, or add-on therapy) in T2DM patients in whom dual therapy with metformin + sitagliptin (MetSita) or metformin + sulfonylurea (MetSu) was initiated.

Materials and methods: Odyssée was a multicenter, longitudinal study. It was conducted in a randomly selected sample of general practitioners in France. Patients were not randomized and were followed for a period of up to three years. Treatments were prescribed at the discretion of the physician. Physicians were expected to include all patients who met the eligibility criteria: Adult (aged ≥ 18 years) patients with type 2 diabetes who had initiated a de novo treatment with metformin and sitagliptin dual therapy or metformin and sulfonylurea dual therapy within the previous eight weeks were eligible for the study. Patient follow-up and changes to treatment were performed according to the physician's clinical practice and were not specified in the study protocol.

Results: At baseline, differences between the two arms (MetSita [$n = 1874$] and MetSu [$n = 733$]) were modest (mean age: 62.4 vs 64.2 years, BMI: 30.3 vs 29.6 kg/m², diabetes duration: 6.4 vs 7 years, respectively). Mean HbA1c levels were similar (7.5 vs 7.6%). The median treatment duration for patients in the MetSita group was longer than the MetSu group (median treatment duration 43.2 vs 20.2 months, respectively; between-group difference 23 months, log-rank $p < 0.0001$). This difference persisted after adjustment for baseline differences and confounders using propensity score methods and application of maximum bias statistical methodology for missing data (42.4 vs 20.2 months). A similar reduction in HbA1c was noted in both arms (-0.6%) and the incidence of hypoglycemia (prior to treatment modification) was lower in the MetSita arm than the MetSu arm (9.7% vs 21.0%). 130 (6.9%) and 58 (7.9%) patients presented a total of 159 and 79 (AEs) during follow-up in the MetSita and MetSu group, respectively. According to the investigating physicians, 60 AEs potentially related to treatment occurred in 52 (2.8%) patients in the MetSita group, and 24 AEs potentially related to treatment occurred in 20 (2.7%) patients in the MetSu group.

Conclusion: Conducted under real-life conditions, the Odyssée study showed that combined therapy with MetSita is maintained without treatment modification longer than combined therapy with MetSu. In addition, the study showed that the glycemic effect is similar, with a lower incidence of symptomatic hypoglycemia, with MetSita compared to MetSu.

Figure 1: Maintenance duration for dual therapy initiated at inclusion for patients receiving metformin + sitagliptin or metformin + sulfonylurea



Supported by: Merck Sharp & Dohme

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Efficacy and safety of a fixed-dose combination of vildagliptin and metformin (LMF237) in Japanese patients with type 2 diabetesM. Yoshiki¹, M. Odawara², M. Sano¹, I. Hamada¹, V. Lukashevich³, W. Kothny⁴;¹Novartis Pharma KK, Tokyo, ²Tokyo Medical University, Japan,³Novartis Pharmaceuticals Corporation, East Hanover, USA,⁴Novartis Pharma AG, Basel, Switzerland.

Background and aims: The use of DPP-4 inhibitor and metformin combination therapy has been increasing in Japanese patients with type 2 diabetes mellitus (T2DM), but no single-pill combination (SPC) of a DPP-4 inhibitor and metformin is currently available in Japan. This clinical study aimed to evaluate the efficacy and safety of an SPC of vildagliptin and metformin (LMF237), in Japanese patients with T2DM inadequately treated with vildagliptin monotherapy. There is limited data assessing the benefit of adding metformin at the doses used in Japan to a DPP-4 inhibitor.

Materials and methods: In a 14-week, multicenter, randomized, double-blind, parallel-group, placebo-controlled study, 171 patients with T2DM were randomized to LMF237 bid ($N=115$) or placebo (vildagliptin monotherapy) ($N=56$) in a ratio of 2:1. In addition, patients in the LMF237 group were further randomized to receive either LMF237 (vildagliptin/metformin) 50/250 mg bid ($N=56$) or 50/500 mg bid ($N=59$) in a 1:1 ratio. The primary efficacy variable included change from baseline to endpoint (Week 14 or final study visit) in HbA1c. The incidence of adverse events (AEs) during the treatment period was also assessed.

Results: Treatment groups were well-balanced for baseline demographic and background characteristics. Patients had a mean age of 57.0 years (73.1% were < 65 years old) and a mean T2DM duration of 7.0 years. A higher percentage of male patients (71.3%) was randomized compared to female patients (28.7%). Mean baseline values of HbA1c, FPG and BMI were 7.9%, 158.7 mg/dL and 25.8 kg/m², respectively. The change in mean HbA1c was -0.83% in the LMF237 group (both doses combined) and 0.14% in the placebo group. The between-group difference in HbA1c change (LMF237 - placebo) of -0.98% was clinically relevant and statistically significant ($p < 0.001$) (Table). In addition, clinically and statistically significant reductions in HbA1c from baseline to endpoint were observed for the subgroups of patients receiving LMF237 50/250 mg bid or 50/500 mg bid (-0.61% and -1.04% , respectively; both $p < 0.001$). The overall incidences of AEs were 43.5% in the LMF237 (both doses combined) and 67.9% in the placebo. In the LMF237 subgroups, the incidence of AEs was similar (44.6% in 50/250 mg bid and 42.4% in 50/500 mg bid). The incidences of serious AEs were low in both treatment groups (0.9% vs 3.6%, respectively). No deaths or hypoglycemic AEs were reported in either group.

Conclusion: Treatment with LMF237 (vildagliptin + metformin SPC) demonstrated a significantly greater reduction in HbA1c compared to placebo (vildagliptin monotherapy), without an increase in hypoglycemia incidence, and was well-tolerated in Japanese patients with T2DM inadequately controlled with vildagliptin alone. Thus, LMF237 is an attractive treatment option for Japanese patients with T2DM.

Table. ANCOVA results for change in HbA1c (NGSP, %) from baseline to endpoint by treatment (FAS)

Treatment	n	Baseline mean (SE)	Adjusted mean change (SE)	Difference in adjusted mean change (LMF - placebo)		
				mean (SE)	(95% CI)	p value
LMF237	115	7.91 (0.08)	-0.83 (0.06)			
Placebo	56	7.97 (0.11)	0.14 (0.08)	-0.98 (0.10)	(-1.17, -0.79)	<0.001

ANCOVA with baseline HbA1c as a covariate. FAS, full analysis set; SE, standard error; CI, confidence interval

Clinical Trial Registration Number: NCT01811485

Supported by: Novartis Pharma KK

PS 071 Non-glycaemic endpoints

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Liraglutide 3.0 mg reduces body weight and improves cardiometabolic risk factors in overweight/obese adults: the SCALE obesity and prediabetes randomised trial

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Background and aims: A 5–10% weight loss has been shown to improve the multiple cardiometabolic comorbidities associated with chronic obesity. This phase 3 trial investigated the effects of liraglutide 3.0 mg, as adjunct to diet and exercise, on weight loss (primary endpoint) and cardiometabolic risk factors (waist circumference, BP, lipids and other biomarkers).

Materials and methods: Individuals (BMI ≥ 27 kg/m² with ≥ 1 comorbidity or ≥ 30 kg/m²) were advised on a 500 kcal/day deficit diet and exercise programme and randomised 2:1 to once-daily s.c. liraglutide 3.0 mg (n=2487) or placebo (n=1244). Randomisation was stratified by prediabetes status (ADA 2010 criteria) and BMI. Data are shown for the full analysis set (exposed individuals with ≥ 1 post-baseline assessment) with LOCF. The trial has an ongoing 2-year extension for participants with prediabetes.

Results: Baseline characteristics were: age 45.1 years, 78.5% female, body weight 106.2 kg, BMI 38.3 kg/m², 61.2% with prediabetes. At week 56, individuals on liraglutide 3.0 mg achieved more weight loss (8.0%) than those on placebo (2.6%) (estimated treatment difference [ETD] -5.4% [95% CI 5.8; -5.0]; $p<0.0001$) (Table). Weight loss was accompanied by reductions in waist circumference (ETD 4.2 [-4.7; -3.7] cm), systolic BP (ETD -2.8 [-3.6; -2.1] mmHg) and diastolic BP (ETD 0.9 [-1.4; -0.4] mmHg) for liraglutide vs placebo (all $p<0.001$). Mean pulse was increased at week 56 with liraglutide 3.0 mg vs placebo (ETD 2.4 [1.9; 3.0] bpm, $p<0.0001$). Improvements in all fasting lipids were seen with liraglutide 3.0 mg vs placebo. Improvements in hsCRP (% relative difference -30% [34; 26]), PAI-1 (-21% [-26; -17]) and adiponectin (+8% [5; 12]) (all $p<0.0001$) were also observed with liraglutide 3.0 mg vs placebo. Liraglutide 3.0 mg reduced net use of anti-hypertensive (OR 1.7, $p<0.0001$) and lipid-lowering (OR 1.5, $p=0.02$) medications at week 56 vs placebo, while maintaining greater beneficial effects on BP and lipids.

Conclusion: Weight loss with liraglutide 3.0 mg, as adjunct to diet and exercise, produced improvements in a wide range of cardiometabolic risk factors, including inflammatory markers, in overweight or obese individuals, which if sustained in the long term may be associated with reduced cardiovascular events.

Table. Effects of liraglutide 3.0 mg on cardiometabolic risk factors after 56 weeks of treatment

Efficacy parameter: Change after 56 weeks	Liraglutide 3.0 mg n=2487 Observed mean (LOCF)	Placebo n=1244 Observed mean (LOCF)	Liraglutide 3.0 mg vs Placebo estimated treatment difference	p value
Body weight (%)	-8.0	-2.6	-5.4 [-5.8; -5.0]	$p<0.0001$
Body weight (kg)	-8.4	-2.8	-5.6 [-6.0; -5.1]	$p<0.0001$
Waist circumference (cm)	-8.2	-3.9	-4.2 [-4.7; -3.7]	$p<0.0001$
Systolic BP (mmHg)	-4.2	-1.5	-2.8 [-3.6; -2.1]	$p<0.0001$
Diastolic BP (mmHg)	-2.6	-1.9	-0.9 [-1.4; -0.4]	$p=0.0009$
Pulse (bpm)	2.5	0.1	2.4 [1.9; 3.0]	$p<0.0001$
Relative change after 56 weeks	Geometric mean (LOCF)	Geometric mean (LOCF)	Liraglutide 3.0 mg vs placebo treatment ratio	p value
Triglycerides (%)	-13.3	-5.5	0.91 (0.88; 0.93)	$p<0.0001$
Total cholesterol (%)	-3.1	-1.0	0.98 (0.97; 0.99)	$p<0.0001$
VLDL-cholesterol (%)	-13.1	-5.5	0.91 (0.89; 0.93)	$p<0.0001$
LDL-cholesterol (%)	-3.0	-1.0	0.98 (0.96; 0.99)	$p=0.002$
HDL-cholesterol (%)	2.3	0.7	1.02 (1.01; 1.03)	$p=0.001$
NEFA (%)	1.6	3.4	0.96 (0.93; 0.99)	$p=0.01$
hsCRP (%)	-37.7	-10.1	0.70 (0.66; 0.74)	$p<0.0001$
PAI-1 (arbitrary units/mL) ^a	12.8	16.1	0.79 (0.74; 0.83)	$p<0.0001$
Adiponectin (%)	11.5	3.0	1.08 (1.05; 1.12)	$p<0.0001$
Fibrinogen (%)	1.0	0.6	1.00 (0.99; 1.02)	$p=0.59$
UACR (%)	12.6	14.8	0.96 (0.90; 1.03)	$p=0.31$

^aData are not adjusted for baseline value as the assay used to analyse the parameter was changed during the trial. hsCRP, high-sensitivity C-reactive protein; PAI-1, Plasminogen activator inhibitor-1; UACR, urine albumin-to-creatinine ratio.

Clinical Trial Registration Number: NCT01272219

Supported by: Novo Nordisk

904

Efficacy and safety of liraglutide 3.0 mg for weight management in overweight/obese adults: the SCALE obesity and prediabetes trial

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Background and aims: Obesity is associated with prediabetes, which is a high risk factor for development of T2D. This trial investigated the effects of liraglutide 3.0 mg, as adjunct to diet and exercise, on weight loss (co-primary endpoints: mean change in body weight [BW] and proportions of individuals losing $\geq 5\%$ and $>10\%$ of BW), safety and tolerability in overweight and obese adults without T2D over 56 weeks.

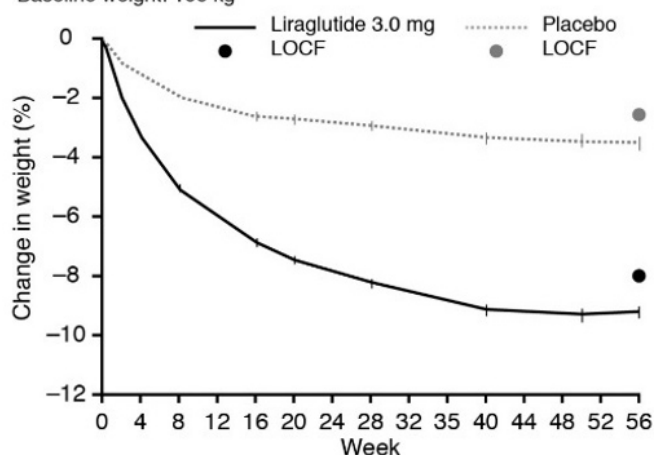
Materials and methods: Individuals (BMI ≥ 27 kg/m² with ≥ 1 comorbidity or ≥ 30 kg/m²) were advised on a 500 kcal/day deficit diet and exercise programme and randomised 2:1 to once daily sc liraglutide 3.0 mg (n=2487) or placebo (n=1244). Randomisation was stratified by prediabetes status (ADA 2010 criteria) and BMI. Data are shown for FAS (exposed individuals with ≥ 1 post-baseline assessment) with LOCF.

Results: Baseline characteristics: age 45.1 \pm 12.0 years, 78.5% female, BW 106.2 \pm 21.4 kg, BMI 38.3 kg/m², 61.2% with prediabetes) completed 56 weeks (71.9% on liraglutide 3.0 mg; 64.4% on placebo). At week 56, individuals on liraglutide 3.0 mg had lost 8.0% (8.4 kg) of BW vs 2.6% (2.8 kg) on placebo (estimated treatment difference [ETD] -5.4% [5.6 kg], $p<0.0001$, ANCOVA) (Fig). Proportion of individuals achieving a BW loss $\geq 5\%$ was 64% with liraglutide 3.0 mg and 27% with placebo (estimated OR 4.8, $p<0.0001$, logistic regression). The proportion achieving BW loss $>10\%$ was 33% and 10%, respectively (OR 4.3, $p<0.0001$). BW loss was independent of prediabetes status at screening (Fig). Consistent with BW loss, liraglutide 3.0 mg also reduced waist circumference (ETD -4.2 cm) and BMI (-2.0 kg/m²) (both $p<0.0001$ vs placebo, ANCOVA), and improved glycaemia, blood pressure and lipids (not shown). The most common AEs with liraglutide 3.0 mg were nausea and diarrhoea, with onset in week 1–4. Most events were mild/moderate and transient. AE withdrawal was 9.9% with liraglutide 3.0 mg (mostly due to gastrointestinal AEs) and 3.8% with placebo. The incidences of gallbladder disorders and pancreatitis were low, although events were reported more frequently with liraglutide 3.0 mg (2.7 and 0.3 events/100 patient years of exposure [PYE], respectively) than with placebo (1.1 and 0.1 events/100 PYE, respectively). Two individuals (1 in each group) co-reported gallbladder-related AEs and pancreatitis.

Conclusion: Liraglutide 3.0 mg, as adjunct to diet and exercise, was superior to placebo on all co-primary endpoints and generally well tolerated.

Figure: Percentage change in weight over time

Baseline weight: 106 kg



Estimated treatment difference liraglutide 3.0 mg vs. placebo at week 56

Total: -5.4 [-5.8 ; -5.0], $p < 0.0001$

Without prediabetes: -5.3 , $p < 0.0001$

With prediabetes: -5.5 , $p < 0.0001$

Test for interaction: 0.59

Observed mean (\pm SE) for participants completing each scheduled visit; full analysis set. Fasting visit data only. LOCF, last observation carried forward.

Clinical Trial Registration Number: NCT01272219

Supported by: Novo Nordisk

905

Liraglutide improves carotid intima-media thickness in patients with type 2 diabetes and non-alcoholic fatty liver disease: an 8-month prospective pilot study

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Background and aims: It has been shown in the last years that GLP-1 analogues, such as liraglutide, have several anti-atherogenic properties beyond their effects on glucose metabolism, including that on subclinical atherosclerosis. Yet, the effects of liraglutide in subjects with non-alcoholic fatty liver disease (NAFLD) are largely unknown.

Materials and methods: We included in a 8-month prospective study 29 subjects with type-2 diabetes and NAFLD (16 men and 13 women, mean age: 61 ± 10 years), who were matched for age and gender with another group of 29 subjects with type-2 diabetes (T2DM) but without NAFLD (16 men and 13 women, mean age: 61 ± 8 years). The diagnosis of NAFLD was based on ultrasonographic and biochemical data. All subjects were naïve to incretin-based therapies and treated with metformin only. Liraglutide was given, on top of metformin, at a dosage of 0.6 mg/day for two weeks, followed by a dose of 1.2 mg/day for the rest of the study. At baseline and every 4 months fasting plasma samples were taken for laboratory analyses and carotid-intima media thickness (IMT) was assessed by B-mode real-time ultrasound. Statistical analysis was performed by ANOVA and the Spearman correlation method.

Results: From baseline to 4 months and 8 months of liraglutide therapy we found significant reductions in HbA1c in both groups of patients (from 8.9 ± 1.5 to 6.6 ± 1.2 to 6.5 ± 1.1 in subjects with T2DM and NAFLD, and from 8.7 ± 0.6 to 7.1 ± 1.1 to 6.9 ± 0.9 in subjects with T2DM only, $p < 0.0001$ for both groups). By contrast, no significant changes were found in body weight, waist circumference, body mass index as well as in plasma lipids for both groups of subjects; yet, it should be noted that some differences approached the statistical significance but probably did not reach it because of the small study groups. Carotid IMT significantly decreased only in the group of patients with T2DM and NAFLD (from 0.96 ± 0.27 to 0.82 ± 0.17 to 0.85 ± 0.12 mm, $p = 0.0325$). Correlation analysis revealed that changes in carotid IMT

after 8 months of therapy were not associated with changes in any other evaluated parameter, including fasting glycemia or HbA1c.

Conclusion: Liraglutide significantly reduced carotid IMT in patients with T2DM and NAFLD, and this beneficial effect was present beyond glycemic control. Yet, whether these findings may translate into a better cardio-metabolic outcome is still unknown.

Clinical Trial Registration Number: NCT01715428

906

The dynamic changes of diabetic maculopathy with intensification of glycaemic control with GLP-1 agonist therapy

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Background and aims: Transient worsening of diabetic retinopathy (DR) with rapid and significant improvement in glycaemic control with GLP-1 agonist therapy has already been well documented. This phenomenon is more common with longer duration of diabetes and pre-existing DR with an inherent risk of maculopathy. The aim of our study was to assess the incidence and outcomes of diabetic maculopathy with sustained GLP-1 therapy in type 2 diabetes.

Materials and methods: A retrospective observational analysis was conducted on all patients who continued on GLP-1 therapy (Exenatide or Liraglutide) beyond 2 years. Retinal screening data at baseline (INITIAL), closest to the lowest HbA1c (INTERIM) and 2 years after initiation (FINAL) were derived from the regional DR screening database. Maculopathy was diagnosed based on referral criteria of UK National screening committee guidelines. HbA1c at baseline, lowest HbA1c achieved on treatment (within the first 12 months) and HbA1c at the latest DR screening were recorded.

Results: 125 patients were included in the analysis. Mean of various parameters were: Duration of diabetes 12.4 years (1–32); Baseline HbA1c 83mmol/mol (45–132); reduction in HbA1c at INTERIM phase was -19 mmol/mol (0 to -70); duration of follow up 933 days (407–1760); HbA1c at FINAL analysis 73mmol/mol (30–143). 82 patients had HbA1c lesser than or equal to their INITIAL value, mean difference being -23 mmol/mol (0 to -68). 72 patients had DR at baseline of whom 49 showing evidence of maculopathy. 3 had resolution of maculopathy at INTERIM study (without Laser) and a further 30 had resolution by the FINAL study (4 of them resolved likely with laser treatment). New onset of maculopathy was noted in 16 patients at INTERIM study. 9 of the 16 patients had no DR at INITIAL study and their duration of diabetes and reduction of HbA1c achieved at INTERIM study was similar to rest of the group. By the FINAL study, maculopathy had spontaneously resolved in 13 of the 16 without any Laser treatment. 9 of the 13 patients had sustained reduction in HbA1c [FINAL HbA1c still being lower than INITIAL HbA1c- mean -34 mmol/mol (-3 to -68)].

Conclusion: Pre-existing maculopathy improves in a significant proportion of patients with improvement in glycaemic control. Worsening of DR with intensification of diabetes control could lead to development of maculopathy but sustained improvement of glycaemic control could potentially reverse this transient complication. Patients with pre-existing DR may be at higher risk of continued progression of maculopathy. The retinal screening programme should have provisions to monitor this paradoxical phenomenon and refer to ophthalmologist if required.

907

Patient-reported outcomes with once weekly dulaglutide 1.5 mg versus once daily liraglutide 1.8 mg (AWARD-6)

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Background and aims: Patient-reported outcomes (PRO) were exploratory objectives in AWARD-6, a noninferiority study designed to compare the efficacy and safety of once weekly dulaglutide (DU), a long-acting GLP-1 receptor agonist, to once daily liraglutide (LIRA) in metformin-treated patients with type 2 diabetes.

Materials and methods: This was a Phase 3, randomised, open-label, parallel-arm study where 599 patients received once weekly DU 1.5 mg or once daily LIRA 1.8 mg for 26 weeks. PRO measures for health status (EQ-5D), impact of weight-related self-perception (IW-SP), and ability to perform physi-

cal activities of daily living questionnaire (APPADL) were administered at baseline and 26 weeks. Analysis of covariance models were fitted with factors of pooled country, baseline score, HbA_{1c} strata (≤ 8.5 , >8.5) and treatment.

Results: DU 1.5 mg was noninferior to LIRA 1.8 mg in HbA_{1c} change from baseline. Significant reductions in weight were observed with both DU 1.5 mg and LIRA 1.8 mg (LS mean [SE]: -2.90 [0.22] and -3.61 [0.22] kg, respectively; $p=0.01$ DU 1.5 mg vs LIRA 1.8 mg). Both groups had significant improvements from baseline in IW-SP, with no between-group differences. Only DU-treated patients had significant improvements from baseline in APPADL ($p=0.014$); between-group differences were not significant. Both groups had significant improvements from baseline in EQ-5D VAS with no between-group differences. In the EQ-5D UK population index score, patients treated with DU 1.5 mg had significant improvements from baseline ($p=0.031$), while there was no significant change with LIRA 1.8 mg and no significant between-group difference.

Conclusion: Both once weekly DU 1.5 mg- and once daily LIRA 1.8 mg-treated patients experienced glycaemic and weight benefits. Significant improvements from baseline were observed in all PRO measures for DU 1.5 mg; significant improvements were only observed in the EQ-5D VAS and IW-SP for LIRA 1.8 mg. There were no significant between-group differences observed in any PRO measures.

	DU 1.5 mg Once Weekly (N=299)		LIRA 1.8 mg Once Daily (N=300)	
Score at 26 weeks	Baseline	Week 26	Baseline	Week 26
Measure, ITT, LOCF	Mean (SE)	LS Mean (SE) ^b	Mean (SE)	LS Mean (SE) ^b
EQ-5D VAS	79.2 (0.88)	82.4 (0.83) [‡]	80.1 (0.85)	81.4 (0.83) [‡]
EQ-5D UK index	0.87 (0.01)	0.90 (0.01) [‡]	0.89 (0.01)	0.89 (0.01)
IW-SP ^a	68.2 (1.69)	74.7 (1.27) [‡]	66.46 (1.67)	75.01 (1.27) [‡]
APPADL ^a	73.56 (1.38)	75.72 (1.07) [‡]	72.64 (1.33)	73.62 (1.07)

Abbreviations: APPADL, Ability to Perform Physical Activities of Daily Living; DU, dulaglutide; EQ-5D, EuroQoL-5 dimension; ITT, intent-to-treat; IW-SP, Impact of Weight on Self-Perception; LIRA, liraglutide; LOCF, last observation carried forward; LS, least squares; SE, standard error; UK, United Kingdom; VAS, visual analog scale. [‡] $p<0.05$ vs baseline. ^aNumber represents the transformed score. ^bLS Mean and SE from Analyses of Covariance.

Clinical Trial Registration Number: NCT01624259

Supported by: Eli Lilly and Company

908

Results of the albiglutide HARMONY program prospective major adverse cardiovascular event (MI, stroke, cardiovascular death, and unstable angina) meta-analysis

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Background and aims: In keeping with guidance issued by the FDA in 2008 requiring demonstration of the cardiovascular (CV) safety of new glucose-lowering agents for type 2 diabetes, GSK conducted a meta-analysis of CV events for albiglutide.

Materials and methods: Possible CV events were collected prospectively across 1 Phase II and 8 Phase III albiglutide studies for blinded adjudication by an independent Clinical Endpoint Committee. Subjects were randomized to albiglutide or to placebo or active comparators (sitagliptin, insulin lispro, insulin glargine, pioglitazone, liraglutide, and glimepiride). Five of the Phase III studies were up to 3 years in duration. There were 5107 subjects in the safety population of whom 2524 were exposed to albiglutide (4870 person-yrs) and 2583 to comparators (5213 person-yrs).

Results: The primary endpoint was the first occurrence of MACE+ (MACE + unstable angina) for albiglutide versus all comparators. MACE (CV death, non-fatal myocardial infarction, or non-fatal stroke) was a pre-defined secondary endpoint. Results are shown in the table.

Conclusion: Although the upper bound of the 95% CI for MACE+ events in this prospective adjudicated metaanalysis was below 1.8, because it is above 1.3, a cardiovascular endpoint study is required for albiglutide. No difference was seen in heart failure events or all-cause mortality for albiglutide versus comparators in this meta-analysis.

Table

Comparison	MACE+ MACE	Hazard Ratio	95% CI (3 year)	NI P Value for 1.8 margin (3 year)	NI P Value for 1.3 margin (3 year)
Albiglutide vs all comparators	MACE+ (116 events)	1.00	0.68, 1.49	0.002	0.10
	MACE (105 events)	0.99	0.65, 1.49	NA	NA
Albiglutide vs placebo	MACE+ (41 events)	0.72	0.38, 1.37	NA	NA
	MACE (37 events)	0.69	0.35, 1.35	NA	NA
Albiglutide vs active comparator	MACE+ (93 events)	1.15	0.74, 1.78	NA	NA
	MACE (83 events)	1.13	0.71, 1.80	NA	NA

Supported by: GSK

909

Safety and efficacy of liraglutide in patients with type 2 diabetes and end-stage renal disease: an investigator-initiated, randomised, placebo-controlled trial

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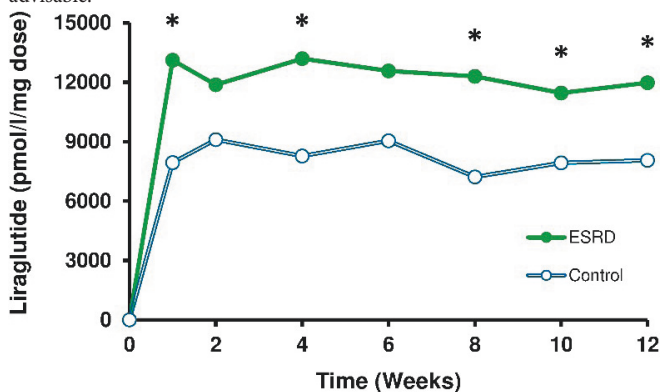
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Background and aims: Diabetes is the leading cause of end-stage renal disease (ESRD), however, few antidiabetic agents are available to these patients. We evaluated safety and efficacy of liraglutide treatment in patients with type 2 diabetes (T2D) and dialysis-dependent ESRD.

Materials and methods: 24 patients with T2D and ESRD and 23 patients with T2D and normal kidney function (71% and 74% treated with insulin, respectively) were randomly allocated to 12 weeks of double-blinded liraglutide (titrated dose of 0.6 mg, 1.2 mg or 1.8 mg) or placebo treatment (1:1) injected subcutaneously once-daily. 20 participants (1:1) in each group completed the study period. Dose-corrected plasma trough liraglutide concentration was evaluated at the final trial visit as the primary outcome measure. Additional safety and efficacy parameters were assessed.

Results: Liraglutide-treated ESRD patients were titrated slower than liraglutide-treated controls, but ended at comparable doses at 12 weeks (1.33 ± 0.13 and 1.26 ± 0.06 mg/day, respectively; $p=0.61$). Dose-corrected plasma trough liraglutide concentrations at the final trial visit were increased by 49% (confidence interval: 6–109%, $p=0.02$) in liraglutide-treated ESRD patients compared with liraglutide-treated controls (plasma concentrations 11,975 [9,379–15,289] and 8,057 [6,306–10,293] pmol/l/mg, respectively) (see Figure). Nausea and vomiting were more frequent in liraglutide-treated ESRD patients compared with liraglutide-treated controls. Severe adverse events were more frequent in the ESRD group ($n=7$, including $n=6$ during liraglutide treatment) compared with controls ($n=1$ during placebo treatment), although none were assessed to be related to the trial medication. Glycaemic control was improved in all treatment arms assessed by HbA_{1c} and blood glucose measurements, and baseline insulin doses were significantly reduced in both liraglutide-treated groups ($p<0.02$).

Conclusion: Liraglutide treatment appears to be applicable in patients with ESRD and T2D. Slower dose escalation and reduced treatment doses may be advisable.



Clinical Trial Registration Number: NCT01394341

Supported by: Novo Nordisk A/S

910

Influence of liraglutide treatment in obstructive sleep apnoea in obese type 2 diabetes patients

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Background and aims: Liraglutide, a long-acting human glucagon-like peptide-1 analogue, is a novel class of type 2 diabetes mellitus (T2DM) treatment that exerts its glucose-lowering effect through different mechanisms that lead to clinically significant reductions in HbA1c and fasting and postprandial glucose levels with moderate weight loss. Concerns about the health impact of obstructive sleep apnoea (OSA) among obese patients with T2DM have been increasing. We aimed to evaluate the impact of liraglutide in the OSA symptoms-somnolence in obese T2DM patients.

Materials and methods: This was a retrospective observational, unicentric study conducted in obese (BMI ≥ 30 kg/m²) T2DM subjects receiving treatment with liraglutide for at least 3 months before study inclusion. Following routine clinical practice, data of the HbA1c, anthropometric measures, blood pressure, and the Epworth Sleepiness Scale (ESS) were collected at the study initiation (V1) and month 3 (V2).

Results: A total of 109 subjects were evaluated (mean age, 59.7 \pm 10.9 years; female, 51.4%). Significant differences between V1 and V2 were found in mean values of body weight (106.0 \pm 16.8 Kg vs 102.3 \pm 15.8 Kg; $p < 0.001$), BMI (40.5 \pm 6.4 kg/m² vs 39.0 \pm 5.9 kg/m²; $p < 0.001$), waist circumference (124.7 \pm 13.5 cm vs 121.4 \pm 12.3 cm; $p < 0.001$), HbA1c (8.2 \pm 1.7% vs 7.4 \pm 1.6%; $p < 0.001$), glycemia (188.4 \pm 48.7 mg/dL vs 164.6 \pm 44.8 mg/dL; $p < 0.001$), fast glycemia (185.6 \pm 81.6 mg/dL vs 135.4 \pm 30.6 mg/dL; $p < 0.005$), total cholesterol (196.2 \pm 52.7 mg/dL vs 173.1 \pm 30.2 mg/dL; $p < 0.05$) and ESS (5.7 \pm 4.7 vs 4.2 \pm 3.5; $p < 0.005$). A significant correlation was observed between baseline ESS score and HDL-cholesterol ($r = -0.248$; $p < 0.05$), Tg/HDL ratio ($r = +0.248$; $p < 0.05$), body weight ($r = -0.215$; $p < 0.05$), and BMI ($r = -0.225$; $p < 0.05$).

Conclusion: Our findings show that treatment with liraglutide significantly reduced the ESS score in obese T2DM patients. Besides this, an improvement in glycemic control, cholesterol and body weight was also achieved.

Supported by: FSEEN

911

Time course and mechanisms of the antihypertensive and renal effects of liraglutide treatment

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Background and aims: Glucagon-like peptide-1 receptor agonist studies have revealed clinically significant reductions in systolic blood pressure (SBP). The aim was to investigate the time course of the anti-hypertensive effect of liraglutide treatment and potential mechanisms behind, in patients with type 2 diabetes.

Materials and methods: Open-label, single-centre trial; 31 patients with type 2 diabetes and hypertension completed the study. All patients were treated with liraglutide up-escalated to maximum dose of 1.8 mg/d for 7 weeks, followed by a 21-day washout period. The primary outcome was change in 24-hour blood pressure.

Results: 24-hour SBP increased by 10 mmHg on day 3 ($p = 0.008$) and 7 mmHg on day 7 ($p = 0.033$, 0.6 mg/d). On day 29, (1.8 mg/d), 24-hour SBP was 7 mmHg lower vs. baseline ($p = 0.106$). Following the treatment period (day 49) and after washout (day 70), 24-hour BP was equivalent to baseline. Secondly, extracellular volume (ECV) was reduced by 2.0 L (CI= 1.0-3.1 L, $p = 0.0005$) and midregional-pro-atrial natriuretic peptide (MR-proANP) by 20% (CI= 12%-28%, $p < 0.0001$). In addition, urinary albumin excretion declined 30% (CI=12%-44%, $p = 0.003$), glomerular filtration rate (GFR) by 11 mL/min/1.73m² (CI=7.2-14.4 mL/min/1.73m², $p < 0.0001$), and fractional albumin excretion by 29% (CI=3%-48%, $p = 0.032$).

Conclusion: Liraglutide treatment was associated with an initial increase in 24-hour SBP, followed by a 7 mmHg reduction after escalation to 1.8 mg/day. This effect subsided after 4 weeks of maximum dose. Reductions in ECV and MR-proANP may explain the antihypertensive potential. Liraglutide treatment was associated with reversible reductions in albuminuria and GFR, which has to be confirmed in randomised blinded trials.

Clinical Trial Registration Number: NCT01499108

Supported by: Novo Nordisk

912

Continuous exenatide infusion improved perioperative glucose control and reduced glycaemic variability in cardiac surgery patients: the EXECUTIVE trial

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Background and aims: Reduction of operation-related hyperglycaemia has been shown to improve postoperative outcomes, though this effect is often blunted by increased number of hypoglycaemic episodes. To intensify perioperative glucose control while minimizing the risk of hypoglycaemia we performed a randomized control trial with the GLP-1 receptor agonist exenatide as add-on to standard perioperative insulin therapy in subjects undergoing elective cardiac surgery.

Materials and methods: Thirty eight subjects (63,2% diabetics) with decreased left ventricular systolic function (ejection fraction $< 50\%$) scheduled for elective CABG (coronary artery by-pass grafting) were randomized to receive either exenatide or placebo in a continuous 72-hour i.v. infusion on top of standard perioperative insulin therapy. Parameters of glucose control and glycaemic variability together with indices of cardiac function assessed by transthoracic echocardiography, perioperative hemodynamic parameters, the need of antiarrhythmic treatment and inotropic drug dosage were collected as primary endpoints.

Results: Compared to placebo group subjects receiving exenatide showed improved perioperative glucose control (average glycaemia 6.1 \pm 2.5 vs. 6.8 \pm 2.8 mmol/l, $p < 0.001$; time in target range of 4.5–6.5 mmol/l 55.0 \pm 3.4 vs. 38.6 \pm 3.3%, $p = 0.001$; time above target range 39.7 \pm 3.3 vs. 53.5 \pm 3.6%, $p < 0.01$) without an increased risk of hypoglycaemia (2 episodes of hypoglycaemia ≤ 3.3 mmol/l in exenatide vs. 4 episodes in placebo group). Exenatide infusion

also reduced glycaemic variability (SD - standard deviation: 1.4 ± 0.5 vs. 2.0 ± 0.6 , $p < 0.01$; MAGE - mean amplitude of glycaemic excursions: 2.5 ± 1.1 vs. 3.3 ± 0.9 , $p < 0.01$) and decreased the need of temporary pacing (16.7 vs. 47.4% of subjects, $p < 0.05$), while no significant difference in perioperative hemodynamics, postoperative echocardiographic parameters and inotropic medication dosage was found between the groups.

Conclusion: Perioperative administration of i.v. exenatide in subjects undergoing elective CABG improved glucose control and decreased glycaemic variability without increasing the risk of hypoglycaemia. Except of decreased need of temporary pacing, exenatide did not significantly affect parameters of cardiac function.

Clinical Trial Registration Number: NCT01373216

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PS 072 Incretin based therapies basic: basic science

913

GLP-1 reduces the activation of PI3K and MAPK pathways and of oxidative stress induced by Na-arachidonate in platelets

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Background and aims: Glucagon-like peptide-1 (GLP-1), exerts not only metabolic but also cardiovascular effects: its influence on platelets, however, is largely unknown. We previously showed that GLP-1 increases platelet sensitivity to the anti-aggregating pathway nitric oxide/cGMP/cGMP-dependent protein kinase (PKG). The GLP-1 influence on pro-aggregating pathways has not been investigated so far. Aim of this study is to verify the GLP-1 ability to interplay with arachidonic acid, a precursor of platelet activators which increases reactive oxygen species (ROS) production: in particular, we aimed at investigating whether the native GLP-1 (7-36), the truncated GLP-1(9-36), and the GLP-1 analogue liraglutide influence the arachidonic acid-induced activation of the signalling pathways PI3-Kinase (PI3K) and MAP-Kinase (MAPK) and of oxidative stress in platelets, and whether these putative GLP-1 effects persist when PKG is inhibited.

Materials and methods: In washed platelets from 24 healthy subjects (M/F 13/11; age 25.6 ± 5.9 years, body mass index 22.5 ± 2.4 kg/m²) we measured the influence of a 15 min pre-incubation with 100nmol/l GLP-1(7-36), or GLP-1(9-36) or liraglutide on the effects of Na-arachidonate (NaA) (0.5 mmol/l) on: i) phosphorylation of Akt and Erk-1/2, molecules of the PI3K and MAPK pathways, respectively (Western Blot); ii) ROS production (DCF-DA assay). Experiments were repeated in the presence of the PKG inhibitor KT5823 (1 micromol/l), the Erk-1/2 inhibitor U0126 (40 micromol/l) and the GLP-1 receptor (GLP-1R) antagonist exendin (9-39) (100 nmol/l).

Results: GLP-1 (7-36), GLP-1 (9-36) and liraglutide, similarly reduced platelet signalling induced by NaA. In particular: i) the fold increase on basal values of pAkt with NaA alone, NaA+GLP-1 (7-36), NaA+GLP-1 (9-36) and NaA+liraglutide was 11.2 ± 2.1 , 3.1 ± 0.8 , 2.8 ± 1.1 , 2.8 ± 1.3 respectively ($p < 0.0001$ vs NaA alone for all), ii) the fold increase on basal values of pErk1/2 with NaA alone, NaA+GLP-1 (7-36), NaA+GLP-1 (9-36) and NaA+liraglutide was 14.6 ± 2.5 , 3.9 ± 1.0 , 3.7 ± 1.5 , 4.0 ± 1.9 respectively ($p < 0.0001$ vs NaA alone for all); iii) in the presence of the PKG inhibitor KT5823 the inhibition of pAkt and pErk-1/2 exerted by GLP-1 (7-36), GLP-1 (9-36) and liraglutide did not occur ($p = ns$ vs NaA alone for all); iv) the fold increase on basal values of ROS with NaA alone, NaA+GLP-1 (7-36), NaA+GLP-1 (9-36) and NaA+liraglutide was 8.2 ± 1.1 , 5.6 ± 2.0 , 5.5 ± 1.1 , 5.5 ± 1.4 respectively ($p = 0.001-0.0005$ vs NaA alone); v) the Erk-1/2 inhibitor U0126 reduced the NaA-induced activation of ROS, its fold increase on basal values with NaA+U0126 being 4.2 ± 1.0 ($p < 0.0005$ vs NaA alone). Pre-incubation with the GLP-1R antagonist exendin (9-39) did not modify the effects of all the incretin preparations on the NaA-induced activation of PI3K and MAPK pathways.

Conclusion: In human platelets, GLP-1 (7-36), GLP-1 (9-36) and liraglutide, independently of GLP-1R, reduce the NaA-induced activation of PI3K and MAPK pathways and of oxidative stress. Because MAPK activation is involved in the NaA-induced increase of oxidative stress, the inhibiting effects of GLP-1 on MAPK activation can account for its ability to attenuate the NaA-induced increase of oxidative stress. Finally, the effects of GLP-1 on intraplatelet signalling do not occur when PKG is inhibited, further confirming the interplay of GLP-1 with PKG.

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Effect of dipeptidyl peptidase-4 inhibitor sitagliptin on CD4⁺ T lymphocytes and monocytes in patients with type 2 diabetesA. Hattori¹, H. Tokuyama¹, F. Shimada², M. Nieda³, Y. Maezawa¹, K. Kobayashi¹, H. Kawamura¹, M. Takemoto¹, K. Yokote¹;¹Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, ²Diabetes and Metabolism, National Hospital Organization Chiba Medical Center, ³Japan Biotherapy Institute, Tokyo, Japan.

Background and aims: Dipeptidyl Peptidase-4 inhibitors (DPP-4i) are currently used widely because of their efficacy and relative low risk of side effects. However several adverse effects of DPP-4i have been reported, e.g. raising the risk of pancreatitis and autoimmune diseases. DPP-4 is identical to CD26, a molecule expressed on immune cells particularly activated helper T cell (CD4⁺T;Th), but the effect of DPP-4i on immune cells is still unclear. The aim of this study is to evaluate the effect of DPP-4i on human T lymphocytes, especially Th, and monocytes *in vivo* and *ex vivo*.

Materials and methods: Thirty-two Japanese patients with type 2 diabetes (19 male and 13 female, mean age 62.5 years, mean HbA1c 7.6%) taking Sitagliptin 50mg/day as the first or the additional medication were enrolled to the study. Blood sampling was performed before and 8 weeks after the treatment. Leukocyte counts were performed by automatic blood cell counter. Surface markers including CD26 and CD163 on lymphocytes and monocytes were measured by flow cytometer after purification of peripheral blood mononuclear cells. *Ex vivo* intracellular cytokine assay was performed to detect subsets of Th: Th0, Th1 and Th2. The concentration of soluble CD26 (=DPP-4), soluble CD163 (macrophage/monocyte activation marker), TNF- α (Th1 cytokine) and IL-4 (Th2 cytokine) in patients' serum were measured by ELISA. **Results:** Cell numbers (T cell numbers/ μ L) and percentages of circulating T lymphocytes were significantly decreased ($1252 \pm 77/\mu$ L vs $1131 \pm 78/\mu$ L, $p=0.02$ and $65.4 \pm 8.9\%$ vs $62.2 \pm 11.3\%$, $p=0.02$) after the administration of DPP4i, although those of total lymphocytes did not change. In the T lymphocyte subset, cell numbers and percentages of Th decreased significantly ($853 \pm 316/\mu$ L vs $764 \pm 286/\mu$ L, $P=0.01$ and $44.8 \pm 8.3\%$ vs $43.0 \pm 9.5\%$, $p=0.04$), but not Tc (CD8⁺T). *Ex vivo* assay showed the significant increase of Th1/2 ratio (8.7 ± 1.0 vs 10.7 ± 1.5 , $p=0.03$) but TNF- α and IL-4 concentrations did not change. The change of Th1/2 ratio in 8 weeks were more correlated to the change of Th2 ($r=-0.90$) than a change of Th1 ($r=0.58$). The number of CD26⁺Th cells and serum level of soluble CD26 did not show any significant change. The percentage of circulating CD163⁺ monocytes (as it is called M2 phenotype monocyte) were significantly decreased ($10.0 \pm 2.3\%$ vs $2.7 \pm 0.5\%$, $p=0.006$) though total monocyte number did not. The concentration of soluble CD163 was significantly decreased ($623 \pm 57\text{ng/mL}$ vs $584 \pm 60\text{ng/mL}$, $p=0.008$).

Conclusion: These findings suggest that DPP-4i possibly effect the balance of circulating T lymphocyte subset, particularly Th, and monocyte, but not effect Th1 and Th2 cytokines. These observation may indicate the direct effect of DPP-4 (CD26) inhibition on the differentiation from Th0 to Th2 rather than to Th1. On the other hand the decrease of soluble CD163 possibly reflects the suppression of macrophage/monocyte activation that could lead to improvement of chronic inflammation. Further detailed study is needed to clarify the effect of DPP-4i to human T lymphocytes and monocytes.

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A dipeptidyl peptidase-4 inhibitor directly suppresses inflammation and foam cell formation in monocytes/macrophages beyond incretins

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Background and aims: We reported that incretins (Glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP)) and a dipeptidyl peptidase-4 inhibitor (DPP-4i) confer an anti-atherosclerotic effect in apolipoprotein-E null mice. It remains unclear whether the anti-atherogenesis of DPP-4i is wholly dependent on incretins or partly initiated via direct mechanisms independent of incretins. In this study we sought to elucidate the direct action of DPP-4i on anti-atherogenesis by examining the *in vitro* effect of incretins and DPP-4i on the expression of inflammatory cytokines and foam cell formation in monocytes/macrophages.

Materials and methods: Mouse peritoneal macrophages were obtained from the ascites by injection of thioglycolate. Monocytes/macrophages were ob-

tained from human peripheral blood mononuclear cells through a CD14 column. Foam cell formation was determined by the incorporation of [³H]-oleate into cholesteryl-oleate stimulated by oxidized-LDL.

Results: Exendin (Ex)-4 (5nM) and GIP (1nM) elicited cyclic (c)AMP generation and suppressed the LPS-induced gene expression of inflammatory molecules such as Interleukin (IL)-1 β , IL-6, TNF- α , and nuclear factor-kappa B by 10~50% in U937 human monocytes. This suppressive effect, however, was attenuated by an inhibitor of adenylate cyclase and mimicked by 8-bromo-cAMP or forskolin. Telenigleptin (1-10 nM) substantially suppressed the LPS-induced expression of inflammatory cytokines by 20~75% without affecting the cell viability or DNA synthesis. Similarly, treatments with antibody or small interfering RNA of CD26 suppressed the expression of inflammatory cytokines. Telenigleptin with DPP4-resistant incretins (Ex-4 and DALa(2)GIP) additively suppressed the expression of these cytokines. Ex-4, GIP, and telenigleptin all suppressed LPS-induced extracellular signal-regulated kinase (ERK) phosphorylation to the same extent. The suppression of p-ERK by incretins was cAMP-dependent, while the suppression by telenigleptin was not. Telenigleptin suppressed cell surface toll-like receptor (TLR)-4 and TLR-5-mediated cytokine production, but had no effect on the intracellular TLRs-induced cytokines. Ex-4 and GIP slightly suppressed foam cell formation in mouse peritoneal macrophages and human macrophages whereas telenigleptin did not. Foam cell formation was enhanced by 2-fold in peritoneal macrophages of diabetic mice (db/db) and in the macrophages of patients with poorly controlled type 2 diabetes. Ex-4, GIP, and telenigleptin suppressed foam cell formation in the macrophages of diabetic mice and patients by 25-50%. The gene expressions of acyl-CoA cholesterol acyltransferase (ACAT)-1 and CD36 were up-regulated in diabetes, and incretins and telenigleptin attenuated the upregulation. CD26 mRNA and transcripts coded by exons 3-13 of the GLP-1 receptor and exons 1-14 of the GIP receptor were detectable in the monocytes/macrophages of mouse and human.

Conclusion: The present *in vitro* study suggests that DPP-4i suppresses inflammation partly via ERK inactivation and foam cell formation by down-regulating CD36 and ACAT-1 in monocytes/macrophages. This suppressive effect appears to be Independent of incretin's action via cAMP.

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Liraglutide associated reversal of obesity and age dependent cardiac fibrosis correlates with improved vascular reactivity and modulation of macrophage gene expressionA.E. Dear¹, T. Gaspari², I. Welungoda², Y. Hu¹, R.E. Widdop², R.W. Simpson¹;¹Medicine, Monash University,²Pharmacology, Monash University, Melbourne, Australia.

Background and aims: Cardiac fibrosis observed in obesity and myocardial aging results from excessive deposition of extracellular matrix (ECM) components, contributes to a detrimental increase in myocardial stiffness and is a major factor in the development of cardiac hypertrophy and the transition to heart failure. Excessive deposition of cardiac ECM is characterised by increased macrophage infiltration, differentiation of fibroblasts to the secretory myofibroblast phenotype and enhanced deposition of type I and III collagens. Expression of the glucagon like peptide-1 receptor (GLP-1R) in cardiac cells together with cardioprotective effects of GLP-1 and GLP-1R agonists in *in vivo* studies suggests a possible role for GLP-1 and GLP-1R agonists in the modulation of cardiac fibrosis. Our current study aimed to determine the effect of the GLP-1R agonist liraglutide in obesity and age induced models of cardiac fibrosis and identify associated molecular mechanisms.

Materials and methods: *In vivo* experiments utilised 5 month old C57Bl/6J mice maintained on a high fat diet (HFD) for 16 weeks to evoke obesity induced hypertension and cardiac fibrosis. 20 month old C57Bl/6J mice maintained on a normal chow diet were utilised as a model of aged induced cardiac fibrosis. Treatment regimens for both obesity and age induced cardiac fibrosis consisted of liraglutide 300 μ g/kg s.c., twice daily for 4 weeks. *In vitro* studies utilised the murine macrophage cell line RAW264.7 stimulated with lipopolysaccharide (LPS) (100ng/ml) and treated with liraglutide (100nM), with or without the specific GLP-1R antagonist exendin-9 (100nM) for 48 hours.

Results: Treatment of 5 month old C57Bl/6J mice with liraglutide prevented HFD-induced increase in blood pressure, reduced fat mass and importantly protected against increased interstitial cardiac fibrosis over vehicle treated animals ($n=4$, $p<0.05$). Studies comparing young 5 month old with 20 month old aged C57Bl/6J mice maintained on a normal chow diet demonstrated

liraglutide treatment significantly inhibited age associated cardiac fibrosis ($n=4-6$, $p<0.05$) and was associated with a reduction in body weight and improved vascular reactivity. In vitro studies identified significant induction of the M2 anti-inflammatory gene IL-10 mRNA expression together with inhibition of the M1 pro-inflammatory gene MCP-1 mRNA expression in liraglutide treated RAW264.7 cells ($n=3$, $p<0.05$).

Conclusion: Taken together these observations identify a potential role for liraglutide in the prevention of obesity and age associated cardiac fibrosis and downstream effects of this condition including cardiac failure in a non-diabetic setting and posits components of a molecular mechanism responsible for these effects.

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The effect of liraglutide and ischaemic postconditioning on myocardial salvage after ischaemia-reperfusion injury in pigs

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Background and aims: Acute ST-segment elevation myocardial infarction (STEMI) is routinely treated by acute primary percutaneous coronary intervention (pPCI) in Denmark. Despite timely reperfusion, this may itself damage the tissue (reperfusion injury). Conditioning with glucagon-like peptide-1 (GLP-1) analogues, used for treatment of type 2 diabetes, has been shown to reduce reperfusion injury. Gradual restoration of blood flow (ischemic postconditioning) provides protection of cardiac tissue following acute myocardial infarction (MI). It has not yet been tested if combined postconditioning treatment with a GLP-1 analogue and ischemic postconditioning offers additive protective effect on the myocardium. We here present preliminary results.

Materials and methods: 58 non-diabetic female Danish Landrace pigs (60±10kg) were randomly assigned to four groups. MI was induced by occlusion of the left anterior descending artery (LAD) for 45 minutes in all groups. Group 1 ($n=14$) was treated with i.v. liraglutide after 15 minutes of ischemia until reperfusion. Group 2 ($n=17$) received liraglutide treatment concomitant with ischemic postconditioning, performed after 45 minutes of ischemia. Group 3 ($n=15$) was treated with ischemic postconditioning, and group 4 ($n=12$) served as controls.

Results: No intergroup differences in relative infarct size were detected (overall mean $57 \pm 3\%$; $p=0.68$). Overall mortality was 34% (CI: 25–41%) including 26% post-intervention, with no intergroup differences ($p=0.99$). Occurrence of VF was 59% (CI: 25–80%) including 39% post-intervention with no intergroup differences ($p=0.65$).

Conclusion: No cardioprotective effects of liraglutide, ischemic postconditioning or combined treatment were found. Thus, based on our preliminary data, we cannot support a class effect of GLP-1 analogues in cardioprotection. Neither an additive effect of additional treatment with ischemic postconditioning could be demonstrated.

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Effects of liraglutide in an adolescent prediabetic transgenic pig model

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Background and aims: To evaluate the effect of the glucagon-like peptide-1 receptor agonist liraglutide in adolescent transgenic pigs expressing a dominant-negative glucose-dependent insulinotropic polypeptide receptor (GIPR^{dn}), with special emphasis to body weight, food intake, glycemic con-

trol, alpha- and beta-cell mass as well as proliferation in the exocrine pancreas.

Materials and methods: GIPR^{dn} transgenic pigs (Trial 1: 2 months, unchanged β -cell mass; Trial 2: 5 months, 35% reduced β -cell mass) were treated with 0.6 mg - 1.8 mg liraglutide or placebo subcutaneously once daily for 90 days. Glucose tolerance was evaluated in a mixed meal oral test. Finally animals were subjected to necropsy and alpha- and beta-cell mass as well as beta-cell and acinus-cell proliferation were determined by quantitative-stereological analyses.

Results: Liraglutide treatment led to marked and sustained reductions in body weight gain (30–40%) and food intake (20–50%) compared to placebo treatment, associated with reduced activation of the growth-related insulin signaling pathway in skeletal muscle. In the mixed meal test performed after the treatment period, liraglutide-treated animals exhibited only a moderate increase of blood glucose, probably due to the known effect of liraglutide on gastric emptying. Additionally, AUC insulin was significantly smaller in liraglutide- vs. placebo-treated animals. Further, liraglutide treatment reduced HOMA-IR and increased several insulin sensitivity indices. Absolute alpha- and beta-cell mass was reduced in liraglutide-treated pigs, but not when related to body weight. Beta-cell proliferation was reduced in liraglutide-treated animals while acinus-cell proliferation in the exocrine pancreas did not differ between the two treatment groups.

Conclusion: The GIPR^{dn} transgenic pig model recapitulates principle clinical effects of liraglutide observed in type 2 diabetic humans. However, the reduction of body weight gain observed in adolescent pigs was much more dramatic than the weight reduction seen in adult patients; thus special care is warranted in prospective treatment trials involving adolescent patients. There was no evidence for an increasing effect of liraglutide on alpha- and beta-cell mass or a stimulating effect on beta-cell and acinus-cell proliferation.

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Exenatide stimulates leptin gene expression and attenuates lipid deposition in skeletal muscle

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Background and aims: GLP-1-based drugs, such as exenatide, exert their beneficial effects on both pancreatic β -cells and extra-pancreatic organs. Here, we aim to assess the stimulation of exenatide on leptin gene expression in adipocytes and determine the effects of exenatide on ectopic lipid deposition in the skeletal muscle in two obese mouse models.

Materials and methods: High fat diet (HFD) fed C57BL/6 mice and db/db mice were treated with exenatide for 4 or 8wks. After treatment, serum leptin level was measured by ELISA kit and lipid accumulation in skeletal muscle was detected by oil red o staining. Key proteins involved in muscle lipid oxidation were determined by Western blotting. Rat primary adipocytes and differentiated 3T3-L1 adipocytes were treated with exendin-4 in vitro. Meanwhile, rat primary adipocytes were transfected with Leptin-LUC fusion gene plasmid by electroporation and LUC reporter activity was measured followed by exendin-4 treatment.

Results: Exendin-4 increased leptin mRNA levels in both rat primary adipocytes and differentiated 3T3-L1 cells, and stimulated leptin gene promoter activity. This increase, however, was not observed in adipocytes from GLP-1R^{-/-} mice. In HFD fed C57BL/6 mice, exenatide administration for four wks resulted in a transient increase of serum leptin levels. In db/db mice, exenatide treatment for 8 wks resulted in increased serum leptin level, associated with increased leptin mRNA expression in epididymal adipocytes and reduced serum triglyceride and free fatty acid contents. In addition, exenatide administration attenuated lipid accumulation in skeletal muscle of db/db mice, along with elevated JAK2 and phosphorylated STAT3 levels, as well as increased expression fatty acid transport protein 1 (FATP1), lipid oxidation related protein CPT1, and lipolysis related protein LPL and ATGL.

Conclusion: This study revealed that adipocytes are among the targets of the incretin hormone GLP-1 and the therapeutic agent exenatide. Our observations support the notion that leptin reduces intramyocellular lipid through activating the short-form leptin receptors.

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Effect of liraglutide on beta cell mass and function in alloxan diabetic mice

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Background and aims: Although GLP-1 receptor agonists have been shown to have beneficial effects on both beta-cell mass and function, the mechanism of the effect of the drugs on beta-cells is still unclear. The objective of this study is to characterize the effect of liraglutide, a long-acting GLP-1 analog, on beta-cell mass and function in alloxan-induced diabetic mice.

Materials and methods: We crossbred Ins2-CreER knock-in mice with R26R-YFP mice to generate Ins2-CreER/R26R-YFP double knock-in mice, in which the pancreatic beta-cells can be labeled specifically and permanently upon injection of tamoxifen. These mice enabled us to discriminate beta-cell neogenesis from self-replication. Pancreatic beta-cells were labeled by 5 consecutive injections of tamoxifen (4 mg/head/injection) to male mice at 6 weeks of age. Ten days after the last injection, diabetes was induced by a single dose of alloxan (60 mg/kg, i.p.). On the following day, mice with blood glucose concentration above 300 mg/dl were used for the study. Liraglutide (200 µg/kg s.c.) or vehicle (saline) was administered once daily from the day following alloxan injection for 30 days. Food intake, body weight, blood glucose levels, serum insulin levels and serum glucagon levels were measured. Beta-cell mass was estimated by immunofluorescent staining of pancreas sections. Proliferation rate was evaluated by Ki67 immunostaining and apoptosis rate was evaluated by TUNEL-method. To investigate beta-cell function, oral glucose tolerance test was performed.

Results: About 80% of beta-cells were destroyed by alloxan injection, leading to hypoinsulinemia and hyperglycemia in the mice. Liraglutide treatment ameliorated hyperglycemia in alloxan-diabetic mice. We found that beta-cell mass in liraglutide-treated group was two-fold higher than that in vehicle group, which correlates with the increment of serum insulin levels. In liraglutide-treated group, proliferation rate was significantly higher and apoptosis rate was significantly lower than in vehicle-treated mice. To examine the effect of liraglutide on beta-cell neogenesis, we injected tamoxifen to the alloxan-treated Ins2-CreER/R26R-YFP mice. As a result, the percentage of YFP-labeled beta-cells was not changed significantly before and after liraglutide treatment, suggesting that the contribution of neogenesis is, if any, limited to the increment of beta cell mass by liraglutide treatment. Furthermore, oral glucose tolerance test showed that the insulin secretory response to glucose was recovered following 30 days liraglutide treatment, and that this effect was sustained two weeks after drug withdrawal.

Conclusion: Chronic treatment of alloxan-induced diabetic mice with liraglutide preserved beta-cell mass by increasing cell proliferation and decreasing cell death. In addition, liraglutide treatment achieved sustained improvement of insulin secretory function of pancreatic beta-cells.

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(ICV) administration of ex4 (250ng) into the third ventricle of the brain for 4 days. Plasma was subsequently collected over a 6 hour time course following i.p. poloxamer administration to prevent lipoprotein uptake. Livers were excised for PCR analysis.

Results: Peripheral ex4 prevented fructose-induced fasting hypertriglyceridemia (vehicle(veh)=3.4 fold triglyceride (TG) increase; ex4=1.4 fold TG increase) and hypercholesterolemia (veh=1.3 fold increase; ex4=0.8 fold decrease). Ex4 also decreased VLDL-triglyceride (1.28mmol/L vs 0.55mmol/L; $p<0.01$), -cholesterol (0.37mmol/L vs 0.16mmol/L; $p<0.05$) and -apoB100 levels following poloxamer (veh=2.4 fold increase; ex4= 0.12 fold increase) indicating a decrease in VLDL production. Ex4 reduced food consumption by 4.3g/day ($p<0.001$) and body weight by 11g over 7 days ($p<0.001$) however pair-fed (PF) hamsters did not significantly differ in fasting lipid levels from vehicles fed ad libitum. Ex4 treated hamsters had decreased expression of hepatic mRNA markers for *de novo* lipogenesis including *srebp-1c* (1.96 fold decrease; $p<0.001$). Ex4 significantly reduced respiratory exchange ratio (veh=0.89; PF=0.90; ex4=0.80; $p<0.001$) and CO₂ production (veh=1355ml/kg/hr; PF=1344ml/kg/hr; ex4=1200ml/kg/hr; $p<0.05$) indicating a switch from carbohydrate to lipid metabolism as the main source of energy utilization. When ex4 was administered by ICV, there was a similar decrease in body weight (10g; $p<0.001$) and food consumption (4g/day; $p<0.05$) however no changes in VLDL-TG, -cholesterol or apoB100 accumulation. This suggests that while the effects of ex4 on satiety may be regulated centrally, the effects on VLDL production may be regulated peripherally.

Conclusion: Our studies suggest that GLP-1R agonism reduces fasting dyslipidemia in insulin resistance by decreasing VLDL production through a peripheral pathway. Additional studies are in progress to examine the involvement of an afferent-efferent signalling pathway. Further experimental evidence to test this role can lead to identification of putative drug targets to fight the metabolic complications of the fasting dyslipidemia observed in T2D.

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Peripheral glucagon-like peptide-1 receptor agonism decreases VLDL production and fasting dyslipidaemia in insulin resistance

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Background and aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone that stimulates pancreatic insulin release to reduce plasma glucose levels and thus acts as an important drug target in the treatment of type 2 diabetes (T2D). While GLP-1 receptor (GLP-1R) agonism is known to enhance satiety and induce weight loss, our laboratory has also shown that GLP-1 regulates lipoprotein metabolism by decreasing intestinal chylomicron production and may also play a similar role in the liver. Hepatic lipids are packaged with apolipoprotein B-100 (apoB100) into VLDL and secreted into the plasma. Dysregulation of VLDL production results in the fasting dyslipidemia that is observed in patients with T2D. In a similar way to intestinal metabolism, we postulate that GLP-1R agonism regulates fasting dyslipidemia by decreasing VLDL production in insulin resistance. We further postulate that this occurs through either a peripheral or central pathway independent of changes in food consumption.

Materials and methods: Fructose-fed insulin resistant Syrian golden hamsters were given twice daily intraperitoneal (i.p.) injections of the GLP-1R agonist exendin-4 (ex4; 20µg/kg) for 7 days and placed into metabolic cages. Alternatively, fructose-fed hamsters received an intracerebroventricular

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Near-normalisation of glycaemic control in patients with type 2 diabetes with a glucagon-like peptide-1 receptor agonist in combination with exercise

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Background and aims: Exercise and glucagon-like peptide-1 receptor agonist (GLP-1RA) treatment, respectively, improve glycaemic control in patients with type 2 diabetes (T2D). We investigated the effects of exercise in combination with GLP-1RA treatment in patients with T2D.

Materials and methods: Thirty-three untrained obese patients with T2D were included in a randomised double-blinded placebo-controlled clinical trial (23 men, age: 56±10 years (mean±SD); weight 99±16 kg; BMI: 32±4 kg/m²; fasting plasma glucose (FPG): 10.1±2.8 mmol/l; HbA1c: 65±14 mmol/mol (8.1±1.3%)), treated with diet only (n=3) or metformin (n=30). Patients were randomised to liraglutide (titrated to 1.8 mg once-daily) and exercise or placebo and exercise during 16 weeks. Both groups had 3 60-minute supervised training sessions per week; 2 spinning sessions and 1 session with resistance training.

Results: HbA1c, FPG and weight reductions were greater with liraglutide and exercise vs. placebo and exercise (Table 1). Both groups exhibited similar reduction in body fat mass and increase in maximal oxygen uptake. The most common adverse event was transient nausea (liraglutide: 24%, placebo: 6%).

Conclusion: Exercise alone for 16 weeks has no or little effect on glycaemic control and body weight, but combined with GLP-1RA treatment it can near-normalise glycaemic control and cause robust weight loss in overweight, dys-regulated patients with type 2 diabetes.

Table 1.

Changes from baseline	Liraglutide + Exercise (n=17)	Placebo + Exercise (n=16)	P value (between groups)
HbA _{1c} (mmol/mol)	-22±14*	-3±10	<0.001
HbA _{1c} (%)	-2.0±1.2*	-0.3±0.9	<0.001
Fasting plasma glucose (mmol/l)	-3.4±2.3*	-0.3±2.6	<0.001
Body weight (kg)	-3.4±2.9*	-1.6±3.2	0.10
Body fat (kg)	-3.4±21.7*	-2.6±2.5*	0.32
Maximal oxygen uptake (l O ₂ per min)	+0.5±0.5*	+0.5±0.4*	0.71

Clinical Trial Registration Number: NCT01455441

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Transfer of liraglutide from blood to cerebrospinal fluid is limited and an unlikely contributor to body weight loss in patients with type 2 diabetes

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Background and aims: The mechanisms by which subcutaneously administered glucagon-like peptide-1 (GLP-1) receptor agonists confer weight loss in humans are incompletely understood. A centrally mediated effect by passage of the blood-brain barrier has been suggested from animal studies. There are no human data on blood-brain transfer of GLP-1 receptor agonists.

Materials and methods: We measured liraglutide in plasma and cerebrospinal fluid (CSF) obtained by lumbar puncture in 8 patients with type 2 diabetes (male: 4/8, age: 63±2 years [mean±SEM], BMI: 30±1 kg/m², HbA1c 56±4 mmol/mol) treated with 1.8 mg liraglutide subcutaneously for at least 1 month and with weight loss 8.4±0.4 kg in response to treatment. Liraglutide was measured with a radioimmunoassay (antibody no. 98302) specific for the intact N-terminus of the GLP-1 moiety of liraglutide. For validation of the CSF-assay, we obtained control CSF from subjects (n=9) not treated with liraglutide.

Results: Plasma-liraglutide was in the nanomolar range (31±4.4 nmol/L). When applying the assay to the CSF control samples, we measured a picomolar baseline level of 17±1.2 pmol/L. Recovery of liraglutide added to CSF was 100 % and sensitivity < 2 pmol/L. The non-corrected CSF-liraglutide concentrations in the liraglutide-treated patients were 23±1.8 pmol/L (comparison to untreated controls, Mann-Whitney test: P0.6).

Conclusion: Blood-brain barrier transport of liraglutide appears very limited and CSF-liraglutide does not seem to correlate with the plasma liraglutide concentrations or with clinical weight loss. Therefore, our results do not support that blood-to-CSF transfer is a significant contributor to the anorectic effects of GLP-1 receptor agonists.

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Exenatide promotes hyperbolic-like specular changes in insulin sensitivity and beta cell function in non-human primates

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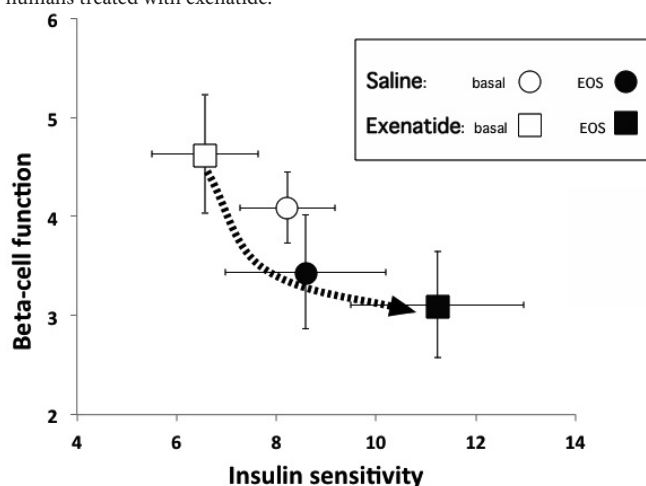
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Background and aims: The GLP-1 receptor agonist Exenatide (EXE) improves glycemic control in type 2 diabetes mellitus (T2DM) by enhancing glucose-dependent insulin secretion, suppressing inappropriate glucagon secretion, slowing gastric emptying, and regulating appetite. However, the effects of continuous chronic EXE infusion on insulin sensitivity and β-cell function are not completely understood. We investigated the effect of continuous EXE infusion on a well characterized non-human primate (baboon) model of insulin resistance and T2DM.

Materials and methods: We examined the effect of a continuous IV infusion (via tether system) of: i) EXE (0.014 ug/kg.h, n=10) or ii) saline (SAL, n=10) on insulin sensitivity and β-cell function in non-diabetic baboons (NDB). At baseline, baboons received a two-step Hyperglycemic Clamp (+125 and +225 mg/dl) followed by an IV Arginine bolus (0.15 g/kg) (HC+A), with a total duration of 210 minutes. Immediately after HC+A, baboons underwent a partial pancreatectomy (~30% total mass) and started EXE or SAL infusion for 13 weeks. At the end of treatment, EXE infusion was stopped for 72 hours before HC+A was repeated and remnant pancreas was collected after humane euthanasia, at necropsy. Insulin sensitivity was calculated by linear regression from the two-step dose-response relationship between the incremental (above baseline) glucose clearance and the incremental insulin concentration measured during the final 30 min of each step. Beta-cell function was calculated by linear regression from the two-step dose-response relationship between the incremental C-peptide concentration and the incremental glucose concentration measured during the final 30 min of each step.

Results: We found a significant increase in insulin sensitivity (6.6 ± 3.4 to 11.2 ± 5.5 10^4 ml/kg per μ U/ml, $p < 0.05$) and a significant decrease in beta cell function (4.6 ± 1.9 to 3.1 ± 1.7 10^2 ng/ml per mg/dl, $p < 0.05$) in the EXE group. No significant changes were found in the saline group. When plotted on the insulin sensitivity vs. beta-cell function diagram (Figure 1), the changes in insulin sensitivity and beta-cell function induced by EXE (Basal vs. End of Study [EOS]) were reminiscent of the hyperbolic-like changes induced by aerobic exercise.

Conclusion: This study shows a significant increase of insulin sensitivity coupled with a significant decrease in beta cell function, after chronic EXE infusion. These results are similar to what has been observed in diabetic patients performing aerobic physical activity. Since baboon is good model to study human metabolic diseases, we speculate that a similar pattern of increased insulin sensitivity and decreased beta function can be reasonably expected in humans treated with exenatide.



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Sitagliptin may improve insulin sensitivity through increases in serum osteocalcin and adiponectin concentrations in patients with type 2 diabetes

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Background and aims: Recent reports have suggested that dipeptidyl peptidase (DPP)-4 inhibitors improve insulin sensitivity in addition to their glucose lowering effects in patients with type 2 diabetes. The underlying mechanisms are not well known. There is growing evidence that osteocalcin, an osteoblast-secreted protein, can enhance insulin sensitivity and secretion in animal models. We aimed to evaluate the relationship between circulating undercarboxylated osteocalcin (unOC) and insulin sensitivity, especially serum adiponectin concentration during sitagliptin add-on therapy in patients with type 2 diabetes.

Materials and methods: A total of 131 Japanese patients with type 2 diabetes were enrolled in the study with the informed consent: 73 males/58 females, age 61 ± 12 years, estimated duration of the disease 11 ± 7 years, body mass index 25.8 ± 4.7 kg/m², and HbA1c 7.9 ± 1.1 %. They were treated with 50 to 100 mg of sitagliptin for 12 months in addition to the preceding anti-diabetic agents. We measured fasting glucose, fasting insulin, indexes of insulin sensitivity (HOMA-IR and quantitative insulin sensitivity check index or QUICKI) and secretion capacity (HOMA- β and C-peptide index or CPI), and serum concentrations of unOC and adiponectin. Next we examined cross-sectional and longitudinal associations between serum unOC and adiponectin.

Results: Sitagliptin add-on therapy significantly improved parameters for glycaemic control (HbA1c: 7.9 ± 1.1 vs. 7.5 ± 1.0 %, $p < 0.0001$) and insulin sensitivity (HOMA-IR: 3.2 ± 2.3 , vs. 2.7 ± 1.8 , $p < 0.0005$, QUICKI: 0.336 ± 0.035 vs. 0.343 ± 0.034 , $p < 0.005$). Serum unOC and adiponectin levels were significantly increased (unOC: 2.22 ± 1.11 vs. 2.48 ± 1.38 , $p < 0.05$, adiponectin: 8.67 ± 5.65 vs. 11.22 ± 8.58 μ g/ml, $p < 0.0001$). Increases in serum adiponectin were significantly associated with decreases in HOMA-IR ($r = -0.228$, $p < 0.05$) and increases in QUICKI ($r = 0.222$, $p < 0.05$). Serum unOC concentrations were positively correlated with adiponectin concentrations after 12-month treatment with sitagliptin ($r = 0.333$, $p < 0.001$) although both parameters showed no association at baseline ($r = 0.103$, $p = 0.2537$).

Conclusion: Sitagliptin improved insulin sensitivity associated with increases in concentrations of unOC and adiponectin, which suggests some potential roles of DPP-4 inhibitors through bone and fat tissues, described as endocrine organs important for the regulation of glucose homeostasis and energy expenditure.

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Effect of lixisenatide vs liraglutide on glycaemic control, gastric emptying and safety parameters in optimised insulin glargine type 2 diabetes mellitus \pm metformin

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Background and aims: The meal test-related pharmacodynamics and safety of lixisenatide 20 μ g and liraglutide 1.2 and 1.8 mg once daily (QD) were compared in this 8-week, randomized, open-label, 3-arm parallel mechanistic trial in patients with type 2 diabetes mellitus (T2DM) \pm metformin after optimal insulin glargine titration.

Materials and methods: The primary endpoint was change from baseline in incremental area under the glucose curve for 4 hours after a standardized solid breakfast (AUC post-prandial plasma glucose [PPG]_{00:30-04:30 h}).

Results: Lixisenatide reduced PPG AUC_{00:30-04:30 h} (least squares [LS] mean \pm standard error) by -240.2 ± 20.0 h.mg/dL, which was significantly more than liraglutide 1.2 and 1.8 mg (-131.8 ± 20.2 and -157.1 ± 21.0 h.mg/dL, respectively; $p < 0.0001$; Table). Lixisenatide delayed gastric emptying more than liraglutide 1.2 and 1.8 mg ($p < 0.0001$; Table) as measured by a ¹³C octanoic acid breath test. A_{1C} decreased significantly from baseline in all groups, with similar reductions with lixisenatide and liraglutide 1.2 mg and small but significantly greater reductions with liraglutide 1.8 mg (by -0.16 %). Mean ambulatory monitored 24-h increase in heart rate was greater with liraglutide 1.2 and 1.8 mg (by 6 beats/min) vs lixisenatide ($p < 0.0001$). Symptomatic hypoglycaemia was slightly more frequent with lixisenatide and liraglutide had more gastrointestinal adverse events reported. There were greater increases in amylase and lipase levels with liraglutide vs lixisenatide (Table) and 1 patient in the liraglutide 1.8 mg group developed asymptomatic oedematous pancreatitis documented by MRI and lipase elevations.

Conclusion: Lixisenatide and liraglutide + insulin glargine improved glycaemic control in T2DM ± metformin, albeit with differing gastric emptying mechanisms of action and safety/tolerability profiles.

Parameter	LIXI 20 µg + IG (n=46)	LIRA 1.2 mg + IG (n=44)	LIRA 1.8 mg + IG (n=46)
AUC PPG _{00:30-04:30 h} , h.mg/dL			
Baseline mean (SD)	282.2 (120.9)	280.1 (99.9)	307.0 (103.2)
Wk 8 (SD)	63.6 (117.9)	171.7 (95.2)	156.7 (62.2)
LS mean change (SE)	-240.2 (20.0)*	-131.8 (20.2)*	-157.1 (21.0)*
LS mean tx difference	—	-108.3	-83.0
LIXI-LIRA [95% one-sided CI]	—	[-140.0] [‡]	[-114.2] [‡]
Gastric emptying <i>t</i> _{lag} , min			
Baseline mean (SD)	113.46 (26.50)	111.23 (19.70)	109.61 (20.84)
Wk 8 mean (SD)	258.85 (145.74)	149.91 (92.21)	125.20 (63.16)
LS mean change (SE)	175.56 (23.71)*	70.10 (23.84) [‡]	48.85 (24.58) [‡]
LS mean tx difference	—	105.46	126.71
LIXI-LIRA [95% CIs]	—	[61.05, 149.87] [‡]	[82.77, 170.64] [‡]
<i>t</i> _{1/2} , min			
Baseline mean (SD)	169.48 (41.07)	161.70 (23.40)	164.30 (27.13)
Wk 8 mean (SD)	537.35 (368.66)	259.23 (216.87)	206.80 (138.36)
LS mean change (SE)	453.56 (58.24)*	175.31 (58.49) [‡]	130.49 (60.27) [‡]
LS mean tx difference	—	278.24	323.07
LIXI-LIRA [95% CIs]	—	[168.71, 387.78] [‡]	[215.27, 430.87] [‡]
A1c%			
Baseline mean (SD)	6.73 (0.39)	6.73 (0.46)	6.85 (0.46)
Wk 8 mean (SD)	6.21 (0.42)	6.14 (0.33)	6.13 (0.33)
LS mean change (SE)	-0.58 (0.06) [‡]	-0.66 (0.06) [‡]	-0.74 (0.06) [‡]
LS mean tx difference	—	-0.08	-0.16
LIRA-LIXI [95% CIs]	—	[-0.20, 0.03]	[-0.27, -0.04] [‡]
24-h heart rate, beats per min			
Baseline mean (SD)	70.0 (10.0)	68.4 (9.8)	69.8 (9.0)
Wk 8 mean (SD)	73.7 (9.0)	78.5 (9.3)	79.3 (8.6)
LS mean changes (SE)	3.3 (1.3) [‡]	9.3 (1.2) [‡]	9.2 (1.3) [‡]
LS mean tx difference	—	6.0	5.8
LIRA-LIXI [95% CIs]	—	[3.7, 8.2] [‡]	[3.6, 8.0] [‡]
Amylase, IU/L			
Mean baseline (SD)	61.27 (24.19)	58.02 (18.41)	58.45 (31.94)
Wk 4 mean (SD)	62.98 (24.82)	71.72 (53.87)	62.26 (28.44)
Wk 4 LS (SE) mean change	-0.47 (6.84)	11.09 (6.74)	0.78 (7.07)
Wk 8 mean (SD)	61.52 (24.84)	64.68 (24.45)	62.50 (27.70)
Wk 8 LS (SE) mean change	2.98 (4.00)	8.01 (4.00) [‡]	5.68 (4.13)
Wk 8 LS mean tx difference	—	5.03	2.70
LIRA-LIXI [95% CIs]	—	[-2.41, 12.47]	[-4.64, 10.04]
Lipase, IU/L			
Mean baseline (SD)	38.98 (17.23)	34.55 (15.78)	39.60 (18.86)
Wk 4 mean (SD)	46.21 (22.95)	60.87 (51.51)	58.30 (30.19)
Wk 4 LS (SE) mean change	1.26 (7.53)	19.61 (7.47) [‡]	11.86 (7.82)
Wk 8 mean (SD)	46.26 (21.13)	55.70 (47.12)	61.20 (42.90)
Wk 8 LS (SE) mean change	6.97 (7.11)	21.12 (7.16) [‡]	20.76 (7.38) [‡]
Wk 8 LS mean tx difference	—	14.15	13.79
LIRA-LIXI [95% CIs]	—	[0.75, 27.54] [‡]	[0.67, 26.92] [‡]

*p<0.0001, [‡]p<0.05 for change vs baseline; [‡]p<0.0001 for change with LIXI vs LIRA; [‡]p<0.0001 vs LIXI; [‡]p<0.05 vs LIXI; [‡]p<0.05 vs LIXI. LIRA: liraglutide; LIXI: lixisenatide; LS=least squares; PPG=post-prandial plasma glucose; SD=standard deviation; SE=standard error.

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Supported by: Sanofi

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The influence of DPP-4 inhibitors on fat metabolism in type 2 diabetes patients

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Background and aims: To evaluate the effect of sitagliptin in combination with metformin on glucose toxicity and lipotoxicity in patients with type 2 diabetes and obesity.

Materials and methods: The study involved 82 patients (55 women, 27 men, mean age 56.1 ± 5.47 years) with obesity, lipid metabolism disorders, who have not reached target levels HbA1c (average HbA1c 8.3 ± 1.6%) after metformin and diet therapy. The first group of patients (42 patients) received co-formulated drug, consisting of sitagliptin 100mg and metformin 2g once a day; the second comparison group (40 patients) received metformin 1.5-2.0 g/day. Dynamics of fasting glycemia, postprandial glycemia, glycated hemoglobin, weight, BMI, WC, WHR, lipid profile (total cholesterol, triglycerides, LDL, HDL, apoB protein), insulin, proinsulin, leptin, adiponectin, insulin resistance using the index HOMA IR and functional activity of β-cells (by HOMA-β index) was evaluated at baseline and at 6 months of therapy. In addition, MRI was performed to assess visceral fat in all the patients.

Results: At 6 months patients in both groups demonstrated significant positive changes in the levels of fasting glucose, postprandial glycemia and glycosylated hemoglobin. In group I, HbA1c decreased from 8.3 ± 1.6% to 6.6 ± 1.24% (p < 0.01), in group II there was a decrease from 8.35 ± 1.75% to 7.62

± 1.39% (p < 0.01). FPG and late products of glycosylation levels in group I reduced on average by 2.67 and 3.3 mmol/L, correspondingly, in group II by 2.1 and 1.8 mmol/L. No significant differences in the dynamics of total cholesterol, HDL between the groups were found. LDL in group I lowered by 0.7 mmol/L, in group II by 0.3 mmol/L (p < 0.05); in group I, TG decreased by 1.33 mmol/L, in group II by 0.63 mmol/L (p < 0.05); in group I IRI reduction was 3.45 mcU/ml, in group II 1.63 mcU/ml (p = 0.05). Proinsulin level dropped down in group I by 2.93 ± 3.02, in group II by 1.26 ± 1.1, C-peptide level increased by 1.4 ± 1.6 ng/ml, in group II 0.16 ± 0.1 ng/ml, HOMA β grew up in group I by 23.4 standard units, in group II by 4.8 standard units (p < 0.005). The ratio of proinsulin/insulin dropped down in group I by 0.19 ± 0.7, in group II by 0.02 ± 0.2. There were no significant differences between the groups in the dynamics of HOMA IR and both groups demonstrated positive dynamics. Adiponectin levels were different between the groups, there was an increase by 1.9 ng/ml in group I, in group II by 0.49 ng/ml. (p < 0.01). Leptin lowered by 7.37 ng/ml in group I, in group II by 1.21 ng/ml (p < 0.01). Also groups showed dramatic difference in anthropometric parameters dynamics (p < 0.001). Average weight loss was 4.9 ± 3.2 kg in group I, in group II 2.0 ± 0.94 kg correspondingly. BMI in group I decreased by 1.8 ± 1.3, in group II by 0.68 ± 0.3. WC shortened by 6.5 ± 4.7 cm in group I, in group II by 2.42 ± 1.02 cm. WHR in group I decreased from 0.95 ± 0.06 to 0.91 ± 0.05 cm, in group II from 0.94 ± 0.03 to 0.93 ± 0.03 cm, respectively. Also MRI showed a significant reduction of visceral fat area by 20.6 ± 13.5 cm² (7.5%) in group I, compared to group II with 5.7 ± 3.75 cm² reduction (1.76%). P < 0.01, while in the dynamics of the area of the subcutaneous fat there is no reliable dynamics between groups. Episodes of hypoglycemia have not been registered in any of the groups during the treatment.

Conclusion: The administration of sitagliptin and metformin decreased glucose toxicity and lipotoxicity that generally led to the improvement of glycaemic control.

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Feed-back suppression of meal-induced GLP-1 secretion: a randomised, prospective comparison of the DPP-4 inhibitors sitagliptin and vildagliptin

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Background and aims: DPP-4 inhibitors inhibit/delay proteolytic degradation of intact incretin hormones (GIP, GLP-1) and thereby stimulate insulin and suppress glucagon secretion. Higher intact GLP-1 increments with DPP-4 inhibitors lead to feedback inhibition of (total) GLP-1 (L-cell-) secretion. In this respect, the present study wanted to compare the effects of vildagliptin and sitagliptin.

Materials and methods: 24 patients with type 2 diabetes (12 without diabetes medication, 12 on metformin monotherapy), including 10 women/14 men, age 63 ± 3 years, BMI 30 ± 4 kg/m², diabetes duration 5 ± 5 years, HbA1c 6.6 ± 0.7 % participated, in randomized order, in treatment periods lasting 7-9 days on either vildagliptin (50 mg b.i.d. = 100 mg/d), sitagliptin (100 mg q.d. in those on diet, 50 mg b.i.d. in those on metformin treatment = 100 mg/d) or placebo (b.i.d.). On the last day of the treatment period, participants underwent a mixed meal stimulation test after an overnight fast. Capillary blood was taken for glucose measurements, venous blood samples were taken for the determination of serum insulin and C-peptide, and plasma GLP-1 and GIP (both intact and total concentrations), and glucagon. Statistics: ANCOVA for repeated measures. **Results:** Both DPP-4-inhibitors significantly reduced the post-prandial increments in plasma glucose. Intact GLP-1 and GIP concentrations were roughly doubled by both DPP-4 inhibitors. Total GLP-1 secretion reduced by 56 % with vildagliptin and by 33% with sitagliptin treatment (significant if compared to placebo, not between vildagliptin and sitagliptin). Glucagon was suppressed slightly more markedly with vildagliptin. Parameters of glycaemic control and insulin secretion did not differ much between vildagliptin and sitagliptin treatment.

Conclusion: Vildagliptin and sitagliptin affect the concentrations of incretin hormones, glucose concentrations, insulin and glucagon secretion as expected, including a feedback inhibition of L-cell secretion induced by DPP-4 inhibition, most likely mediated by elevated „intact“ GLP-1 concentrations. In this respect, and regarding more clinical parameters (glycaemic control, insulin secretion, glucagon suppression) at most minor differences were described between vildagliptin and sitagliptin treatment, which most likely are without clinical significance.

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Effects of standard dose of vildagliptin vs sitagliptin on blood glucose fluctuations evaluated by long-term self-monitoring blood glucose (SMBG)

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Background and aims: No previous studies have compared the DPP-4 inhibitors, vildagliptin and sitagliptin, in terms of fluctuations of daily blood glucose profile by using long-term SMBG. The aim of this study was to evaluate the effects of switching from sitagliptin to vildagliptin, or from vildagliptin to sitagliptin on 12-week glucose fluctuations using SMBG in Japanese patients with type 2 diabetes.

Materials and methods: We conducted a multicenter, prospective, open-label controlled trial with blinded endpoint analysis. Thirty-two patients with type 2 diabetes mellitus had been treated by vildagliptin or sitagliptin in each standard use (vildagliptin 50mg twice daily or sitagliptin 50mg once daily), and were allocated to two groups who received vildagliptin during 12 weeks then switched to sitagliptin during 12 weeks, or vice versa. SMBG systems were distributed to all patients at the beginning and measurements of seven-point SMBG (daily profile of blood glucose level of before and 2 hours after each meal, and at bedtime) during the trial were used to determine: 1) the mean amplitude of glycemic excursions (MAGE), 2) M-value, 3) mean (\pm standard deviation) blood glucose level, and 4) fasting and postprandial blood glucose level. Plasma glycosylated hemoglobin (HbA1c), glycoalbumin (GA), 1,5-anhydroglucitol (1,5AG), immunoreactive insulin (IRI), Proinsulin/IRI ratio, C-peptide immunoreactivity (CPR) and glucagon levels were measured.

Results: The MAGE was significantly lower in patients taking vildagliptin than sitagliptin (61.9 ± 27.3 vs. 72.9 ± 38.4 mg/dL; $p=0.04$). In patients taking vildagliptin, the mean blood glucose level was significantly lower (151.0 ± 31.6 vs. 157.5 ± 29.2 mg/dL; $p=0.02$), M-value was significantly lower (11.7 ± 2.4 vs. 14.5 ± 2.5 ; $p=0.01$) and the blood glucose level after supper was significantly lower (172.2 ± 40.8 vs. 190.6 ± 42.2 mg/dL; $p=0.04$). 1,5-anhydroglucitol (1,5AG) was significantly higher (1.40 ± 2.67 vs. -0.90 ± 1.59 ; $p=0.003$), and proinsulin/IRI ratio was significantly lower (-0.039 ± 0.13 vs. 0.51 ± 0.11 ; $p=0.01$). There were no significant differences in plasma HbA1c, GA, fasting CPR, or glucagon levels between patients taking vildagliptin and sitagliptin.

Conclusion: Vildagliptin was superior to reduce the fluctuations of daily blood glucose profile compared with sitagliptin in Japanese patients with type 2 diabetes.

Clinical Trial Registration Number: UMIN000005627

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Factors associated with progression of type 2 diabetes and impact of treatment with saxagliptin in the SAVOR-TIMI 53 trialG. Leibowitz¹, O. Mosenzon¹, D.L. Bhatt², B. Hirshberg³, C. Wei³, A. Cahn¹, G. Jermendy⁴, W.H.H. Sheu⁵, J. Lopez-Sendon⁶, A. Avogaro⁷, B.M. Scirica², I. Raz¹;

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Background and aims: In type 2 diabetes (T2D), glycemic control often deteriorates over time, requiring intensification of treatment. We aimed to identify factors associated with progression of diabetes and studied the impact of saxagliptin, a DPP-4 inhibitor, on diabetes progression. In addition, we evaluated the effect of saxagliptin on beta cell function as reflected by a decline in HOMA2- β .

Materials and methods: We studied the association of clinical and biochemical parameters with diabetes progression in the SAVOR-TIMI 53 study, a randomized clinical trial of 16,492 patients with T2D treated with saxagliptin vs. placebo added to current anti-diabetic medications for a median of 2.1 years. Diabetes progression was defined by 1) HbA1C increase $\geq 0.5\%$, 2) initiation of new anti-diabetic medications, 3) increase in oral medication dose or 4)

$\geq 25\%$ increase in insulin dose for ≥ 3 months. HOMA2- β was measured at baseline and at year 2 in 4134 patients (25.1% of trial).

Results: Progression of diabetes during the study occurred in 54.7% of all subjects. Compared with placebo, treatment with saxagliptin decreased the risk of diabetes progression (OR 0.60; 95% CI 0.57–0.65; $p<0.001$). The occurrence of an HbA1C increase of $\geq 0.5\%$, initiation of insulin, and the increase in dose for an oral hypoglycemic medication or insulin was decreased by 30%, 30% and 19% respectively in patients treated with saxagliptin compared with placebo. At 2 years, HOMA2- β was decreased by 7.6% with placebo, compared with 2.7% with saxagliptin ($p=0.0004$). A multivariate analysis that included baseline demographics, biochemical parameters, and medical treatments showed that older age, lower HDL, lower baseline HOMA2- β , and baseline sulfonylurea use were significantly associated with diabetes progression.

Conclusion: Saxagliptin decreased the progression of diabetes via improved glycemic indices and fewer concomitant anti-hyperglycemic agents compared with placebo, which may be related to reduced natural decline in β -cell function.

Clinical Trial Registration Number: NCT01107886

Supported by: AstraZeneca/Bristol-Myers Squibb

PS 074 The potential future of insulin therapy

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IDegAsp provides superior FPG control and reduced hypoglycaemia vs BAsp 30 in insulin-naïve adults with type 2 diabetes: a randomised phase 3 trial

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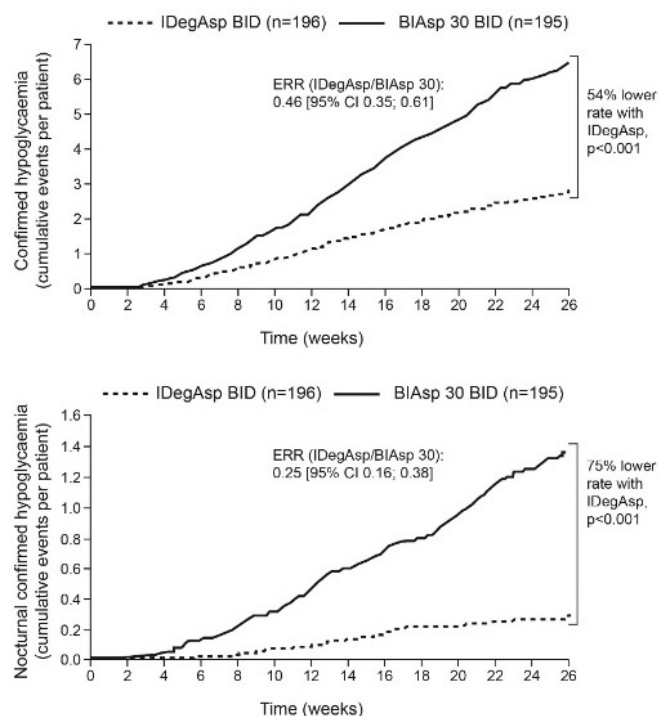
Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a 70%/30% combination of insulin degludec (IDeg) and insulin aspart (IAsp), providing both a basal insulin with an ultra-long duration of action and a rapid-acting bolus insulin in a single injection. The aim of this trial was to compare the efficacy and safety of IDegAsp and biphasic insulin aspart 30 (BAsp 30), each administered twice daily (BID) \pm metformin in insulin-naïve adults with type 2 diabetes (T2DM).

Materials and methods: This was a 26-week randomised, open-label, multinational, treat-to-target trial. Subjects (mean: age 58.9 years, duration of diabetes 9.5 years, HbA_{1c} 8.4%, FPG 10.2 mmol/L, BMI 31.2 kg/m²) were randomised 1:1 to IDegAsp BID (n=197) or BAsp 30 BID (n=197). Insulin was administered before breakfast and the main evening meal and titrated to a pre-breakfast and pre-main evening meal self-measured plasma glucose (SMPG) target of ≤ 5 mmol/L. Patient characteristics were similar in both treatment arms.

Results: IDegAsp was non-inferior to BAsp 30 based on mean change in HbA_{1c} from baseline (primary endpoint; estimated treatment difference [ETD] +0.02% [95% CI -0.12; 0.17]), end of treatment mean HbA_{1c} was 49.0 (6.6) and 48.0 (6.5) (mmol/mol [%]), respectively. IDegAsp was superior to BAsp 30 in lowering FPG (ETD -1.0 mmol/L [95% CI -1.42; -0.59]; $p < 0.001$). Mean daily insulin dose after 26 weeks was similar in both treatment arms. IDegAsp was associated with significantly lower rates of confirmed (plasma glucose (PG) < 3.1 mmol/L or severe [requiring assistance]) and nocturnal confirmed (between 00.01–05.59 h) hypoglycaemia vs BAsp 30 (Figure). Rates of severe hypoglycaemia were low in both treatment arms. Both treatments were well tolerated and rates of adverse events were similar.

Conclusion: IDegAsp BID in insulin-naïve adults with T2DM effectively improves HbA_{1c} (non-inferior to BAsp 30) and is superior to BAsp 30 in lowering FPG. The significantly lower rates of confirmed and nocturnal confirmed hypoglycaemia for IDegAsp vs BAsp 30 are consistent with previous findings, reflecting the more prolonged and stable glucose-lowering effect of the IDeg (basal) component in IDegAsp compared to the protaminated portion of IAsp in BAsp 30.

Rates of confirmed and nocturnal confirmed hypoglycaemia



ERR, estimated rate ratio

Clinical Trial Registration Number: NCT01513590

Supported by: Novo Nordisk A/S

932

Pharmacokinetic (PK) and pharmacodynamic (PD) properties of BioChaperone Combo (BC Combo), the first fixed combination of glargine and lispro, in type 1 diabetes (T1DM)

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Background and aims: BC Combo is a novel insulin formulation in a clear solution combining the two already licensed insulin analogues glargine and lispro (75/25) at neutral-pH using the BioChaperone technology.

Materials and methods: We investigated the pharmacodynamic and pharmacokinetic properties in patients with T1DM (21 randomised (mean \pm SD age: 42.3 \pm 13.0 years; HbA_{1c}: 7.8 \pm 0.6 %), 19 completers) receiving a single dose of 0.8 U/kg (s.c.) of BC Combo or Humalog Mix25 (MIX) under automated glucose clamp conditions (ClampArt[®], target blood glucose (BG) 100 mg/dL, duration 30 h post-dosing) in this double-blind, 2 way-crossover study.

Results: As visible from the mean glucose infusion rate (GIR) curves (Figure), BC Combo showed a faster onset of action (25 \pm 11 vs. 40 \pm 13 min, $p = 0.0017$), an earlier T_{max} (2.8 \pm 0.8 vs. 3.4 \pm 0.8 h, $p = 0.0145$) and an improved glucose-lowering effect in the first two hours post-dosing ($AUC_{GIR\ 0-2h}$ 504 \pm 210 vs. 325 \pm 183 mg/kg, $p = 0.0012$), compared with MIX, likewise evidenced by a greater early appearance ($AUC_{INS\ 0-1h}$ 86 \pm 39 vs. 34 \pm 19 h* μ U/L, $p < 0.0001$). Minimal duration of action was achieved before 30 hours by less subjects on BC Combo than on MIX (2 vs 14, $p = 0.0002$) indicating a longer duration of action (defined as the time after dosing at which the BG increases to > 118 mg/dL, Figure) for BC Combo (29.8 \pm 0.7 vs. 25.5 \pm 4.3 h). This also resulted in lower mean BG values in the last hour of the clamp (104.5 \pm 8.8 and 137.8 \pm 35.7, $p = 0.0008$). In addition, BC Combo showed a more pronounced late metabolic effect ($AUC_{GIR\ 12-30h}$ 1480 \pm 900 vs. 961 \pm 553 mg/kg, $p = 0.0257$) which was in line with a higher late exposure ($AUC_{INS\ 12-30h}$ 563 \pm 409 vs. 286 \pm 233 h* μ U/L, $p < 0.0001$) and a longer half-life (17.6 \pm 8.7 vs. 7.7 \pm 3.0 h, $p < 0.0001$) supporting the use of BC Combo as a once daily injection. No

safety issues were identified. Both formulations were well tolerated and no injection site reactions occurred.

Conclusion: The PK and PD results of BC Combo showed a stronger prandial effect with a faster onset and also a longer basal action for BC Combo indicating the potential of an improved metabolic control vs. MIX when used as once daily treatment.

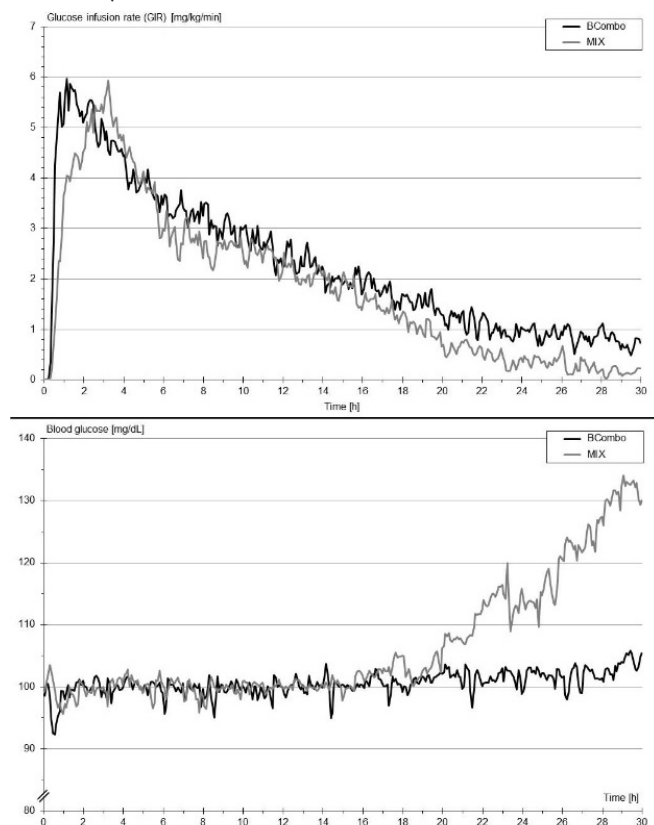


Figure 933: Pharmacodynamic (GIR, upper panel) and blood glucose (BG, lower panel) profiles (5 min mean values, respectively) of BioChaperone Combo and Humalog Mix25

Clinical Trial Registration Number: NCT01981031

933

Long-acting basal insulin (HM12470) offers once-weekly dosing potential with a favorable PK, PD and mitogenic profile

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Background and aims: The novel long-acting basal insulin (HM12470) has been developed for once-weekly injection by conjugating an insulin analog with the constant region of a human immunoglobulin fragment via non-peptidyl linker. This study investigated the *in vitro* properties, pharmacokinetics and pharmacodynamics of HM12470 in normal and diabetic animal models to evaluate the once-weekly dosing potential and the mitogenic potency.

Materials and methods: Pharmacokinetics of HM12470 was evaluated after subcutaneously administration to mice, rats, dogs, pigs, and monkeys. Based on these preclinical PK data, human plasma profile of HM12470 was predicted for once-weekly dosing by the Wajima C_{ss} -MRT method. Blood glucose lowering was monitored in *db/db* mice after HM12470 was subcutaneously injected. *In vitro* analysis, the lipogenic potency in fully differentiated 3T3-L1 adipocyte cells, and cell proliferative potency in MCF-7 and SaOs-2 cells were evaluated.

Results: In a pharmacokinetic study, subcutaneously injected HM12470 exhibited a half life of ~ 43 hr in normal rats, while insulin degludec showed 2.9 hr of half-life. The extended half-life was also confirmed in other species such as mice, dogs, pigs, and monkeys. The improved pharmacokinetic profile had contributed to prolonged glucose lowering efficacy in *db/db* mice. Based on the results from three different species (mice, rats, and dogs), human phar-

macokinetics was predicted that half-life time of HM12470 was expected to be ~132 hr and the peak-to-trough ratio was to be 1.6 on once weekly dosing. *In vitro* mitogenic potency of HM12470 was assessed by using cell proliferation in MCF-7 and SaOs-2 cells. Compared to its lipogenic efficacy assessed in fully differentiated 3T3-L1 adipocyte cells, the mitogenic to lipogenic potency ratio was significantly lower than that of human insulin.

Conclusion: HM12470 has a prolonged pharmacokinetic and pharmacodynamic profile in animal studies, and the predicted half-life and peak-to-trough ratio of HM12470 in human are promising once-weekly dosing potential with flat exposure profile. Moreover, HM12470 is expected to have low mitogenic potency based on *in vitro* results.

934

The ultra-rapid biochaperone insulin lispro shows a faster onset of action and stronger early metabolic effect than insulin lispro alone

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Background and aims: We investigated the pharmacodynamic characteristics of BioChaperone insulin lispro (BC LIS), a novel insulin lispro formulation with BioChaperone aimed at accelerating the absorption from the subcutaneous tissue.

Materials and methods: In this double-blind, crossover study people with type 1 diabetes received 0.2 U/kg of BC LIS or insulin lispro (LIS) alone under automated glycaemic clamp conditions (ClampArt®, target blood glucose 100 mg/dL, clamp duration 6h post-dosing).

Results: Thirty-seven patients were enrolled and 36 completed this study (age 42.7±13.4 years (mean±SD), BMI 24.8±1.7 kg/m², HbA1c 7.6±0.6%). Mean glucose infusion rates (GIR) are given in the figure. Compared with LIS, BC LIS showed ultra-rapid properties with a faster onset of action (23.1±7.0 vs. 34.4±15.3 min, $p<0.0001$), an earlier maximum effect ($T_{GIR\ max}$ 99±42 vs. 133±45 min, $p=0.0002$) and a stronger early metabolic effect in the first hour ($AUC_{GIR\ 0-1h}$ 218±88 vs. 129±63 mg/kg, $p<0.0001$) and first 2 hours ($AUC_{GIR\ 0-2h}$ 627±235 vs. 525±214 mg/kg, $p=0.0041$). Total ($AUC_{GIR\ 0-6h}$ 1409±494 vs. 1434±506 mg/kg, $p=0.72$) and maximum metabolic effect (GIR_{max} 7.85±2.87 vs. 7.96±2.81 mg/kg/min, $p=0.76$) were comparable. Both insulin formulations were well tolerated.

Conclusion: BC LIS has the characteristics of an ultra-fast acting insulin with a faster onset of action, an earlier maximum action and stronger metabolic effect in the first 2 hours than insulin lispro alone. BC LIS therefore has the potential to provide excellent postprandial glycaemic control.

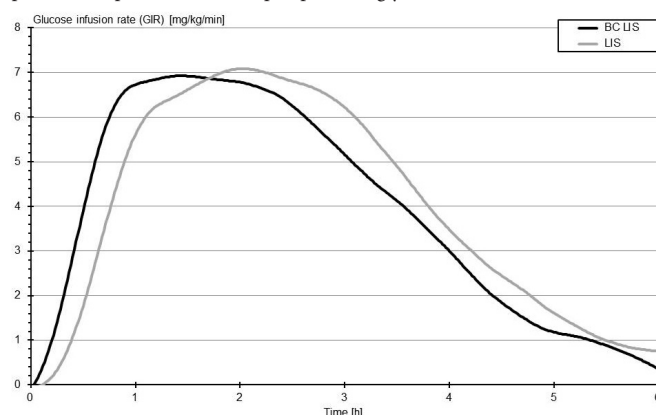


Figure 934: Smoothed pharmacodynamic (GIR) profiles of BioChaperone lispro (BC LIS) and native insulin lispro (LIS)

Supported by: 2013-003507-19

935

Comparison of duration of action of 2 insulin glargine products, LY2963016 and insulin glargine, in subjects with type 1 diabetes mellitus
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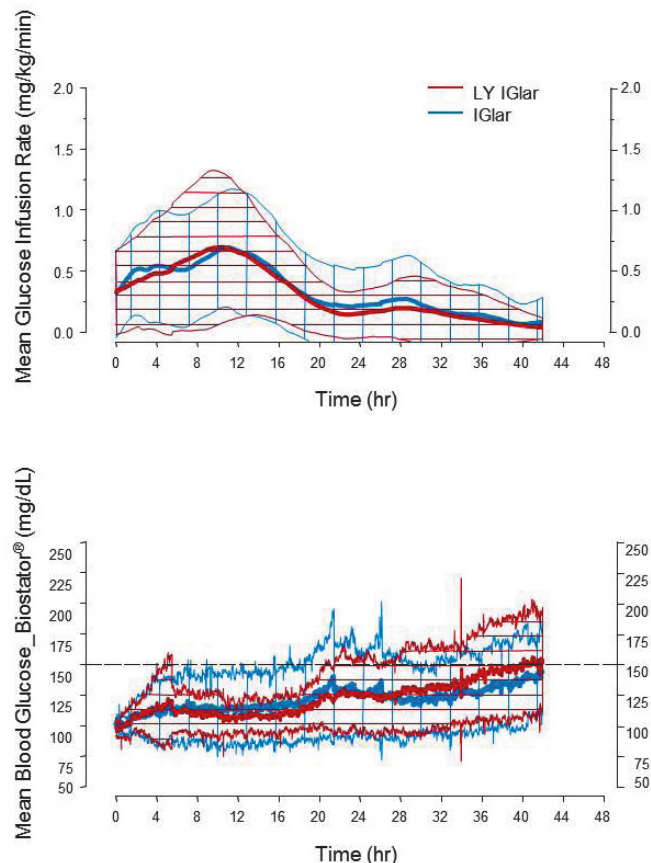
Background and aims: LY2963016 (LY IGLar) and insulin glargine (Sanofi-Aventis; IGLar) are both insulin glargine products, with identical amino acid sequences. Even with identical amino acid sequences, proteinbased therapeutics manufactured by distinct processes must be shown to be similar. This study compared the duration of action of LY IGLar and IGLar.

Materials and methods: This was a Phase 1, randomised, doubleblind, 2period, crossover, glucose clamp study. Fasted male subjects with type 1 diabetes mellitus received a single subcutaneous dose of 0.3 U/kg LY IGLar and IGLar on 1 occasion each. A minimum 7day washout separated doses. Pharmacodynamic effects were assessed with a euglycaemic clamp (Biostator®, glucose target level 100 mg/dL [5.6 mmol/L]) lasting up to 42 hours postdose. Duration of action was defined as the time post dosing at which the subject's blood glucose level was consistently >150 mg/dL (8.3 mmol/L) without any glucose infusion.

Results: Twenty male subjects aged 23.54 years with type 1 diabetes mellitus participated in this study. Based on timetoevent (survival) analysis, median duration of action was estimated to be 37.1 and 40.0 hours for LY IGLar and IGLar, respectively, while mean duration of action (standard error) was 23.8 (3.8) and 25.5 (3.9) hours for LY IGLar and IGLar, respectively. In 14 of the 40 clamps (7 LY IGLar, 7 IGLar), subjects' duration of action exceeded the 42hour clamp period. The mean profiles of the pharmacodynamic variables (glucose infusion rate and blood glucose) were similar between treatments over the 42hour clamps (see figure below). LY IGLar was well tolerated, with similar adverse event profiles observed following LY IGLar and IGLar dosing. No safety concerns from clinical laboratory, vital signs or ECG data were noted.

Conclusion: Similar pharmacodynamic effects and duration of action were demonstrated for LY IGLar and IGLar in this 42hour clamp study in subjects with type 1 diabetes mellitus.

Figure: Mean (and 90% confidence interval) glucose infusion rate-time profiles (upper) and corresponding Biostator® glucose levels (lower) following a single subcutaneous administration of 0.3 U/kg LY IGLar (LY2963016) or IGLar (insulin glargine)



Clinical Trial Registration Number: NCT01600950

Supported by: Eli Lilly and Company

936

Higher early insulin exposure and greater early glucose-lowering effect with faster-acting insulin aspart in patients with type 1 diabetes mellitus

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Background and aims: Faster-acting insulin aspart (faster aspart) is insulin aspart (IAsp) in a new formulation containing two new excipients (nicotinamide and arginine), which result in a faster initial absorption after s. c. injection.

Materials and methods: In total, 52 patients with type 1 diabetes mellitus (mean ± standard deviation age: 40.3 ± 12.0 years; haemoglobin A_{1c}: 7.3 ± 0.7%) were randomised to a single dose (0.2 U/kg s. c.) of faster aspart or IAsp under glucose clamp conditions (Biostator; blood glucose target 100 mg/dl; clamp duration 12 hours post-dose) in a crossover design.

Results: Compared with IAsp, faster aspart had a faster onset of appearance, i.e., time from trial drug administration until the first time serum insulin aspart concentrations reached the lower limit of quantification (mean difference [95% confidence interval (CI)]: -6.33 minutes [-7.30; -5.36]), earlier time to 50% C_{max} (median difference [95% CI]: -11.0 minutes [-13.5; -9.0]), earlier t_{max} (median difference [95% CI]: -7.5 minutes [-17.5; 0.0]), and greater early exposure up to 2 hours post-dose (e.g., 4.5-fold more insulin aspart in the circulation in the first 15 minutes post-dose); total exposure was similar (Table). Faster aspart had an earlier and higher glucose-lowering effect (indicated by higher glucose infusion rates, GIR) in the first 2 hours post-injection versus IAsp (Table) and an earlier time to 50% GIR_{max} (mean difference [95% CI]: -7.81 minutes [-13.19; -2.44]). The maximum GIR and total glucose-lowering effect (Table) were similar between faster aspart and IAsp. No safety or tolerability issues were identified; in particular, no injection site reactions occurred.

Conclusion: Faster onset and higher early exposure with faster-acting insulin aspart led to a greater early glucose-lowering effect, indicating that faster aspart has the potential to improve postprandial glucose versus IAsp.

Table: PK and PD results for faster-acting insulin aspart versus insulin aspart.

PK endpoints	Insulin exposure [†] Ratio [95% CI]	PD endpoints	Ratio [95% CI]
AUC _{0–15 minutes}	4.53 [3.62; 5.66]	N/A	N/A
AUC _{0–30 minutes}	2.05 [1.76; 2.38]	AUC _{GIR, 0–30 minutes}	1.48 [1.13; 2.02]
AUC _{0–1 hour}	1.28 [1.15; 1.43]	AUC _{GIR, 0–1 hour}	1.31 [1.18; 1.46]
AUC _{0–1.5 hours} [§]	1.11 [1.01; 1.22]	AUC _{GIR, 0–1.5 hours}	1.17 [1.05; 1.30]
AUC _{0–2 hours}	1.04 [0.95; 1.14]	AUC _{GIR, 0–2 hours} [‡]	1.10 [1.00; 1.22]
C _{max}	0.98 [0.90; 1.07]	GIR _{max}	1.02 [0.93; 1.12]
AUC _{0–12 hours}	0.96 [0.87; 1.06]	AUC _{GIR, 0–12 hours}	0.98 [0.87; 1.11]

[†]Based on free serum insulin aspart; [‡]Primary endpoint; [§]Post hoc endpoint; Ratio: faster-acting insulin aspart/insulin aspart; AUC=area under the curve; GIR=glucose infusion rate; PD=pharmacodynamics; PK=pharmacokinetics.

Clinical Trial Registration Number: NCT01618188

Supported by: Novo Nordisk

937

Biphasic pharmacokinetic and pharmacodynamic profiles associated with concentrated insulin BIOD-531 show rapid onset and basal duration of action

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Background and aims: Formulations of human insulin and insulin analogs containing citrate and EDTA have been shown to be more rapidly absorbed than commercially available insulin products. The aim of this study was to compare the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of BIOD-531, a concentrated (400 U/ml or U-400) formulation of recombinant human insulin containing EDTA, citrate and MgSO₄, to products marketed as combined prandial/basal insulins.

Materials and methods: A single-center, randomized, double-blind four-period crossover study employing 24-hour euglycemic clamps, evaluating the PK and PD profiles of BIOD-531 at two doses (1 U/kg and 0.5 U/kg) vs. Humulin® R U-500 (1 U/kg, U-500R) and Humalog® Mix75/25™ (0.5 U/kg, HU 75/25) in obese non-diabetic subjects.

Results: Thirteen subjects (age 39.8 ± 13.5 yrs; weight 99.5 ± 12.6 kg) were randomized. BIOD-531 was associated with an increased rate of absorption vs. U-500R as measured by multiple PK parameters including a 92% shorter time to early ½ T_{max} (11.0 ± 1.9 min) vs. U-500R (135.3 ± 34.9 min), a 43% shorter time to T_{max} (223.8 ± 62.3 min) vs. U-500R (393.3 ± 58.3 min) and a 765% increase in early insulin exposure as measured by AUC_{ins0–30min} (2966 ± 383 mU*min/L) vs. U-500R (343 ± 74 mU*min/L). BIOD-531 was associated with a more rapid glucose lowering effect as measured by multiple PD parameters including a 66% faster time to onset of action (7.2 ± 4.1 min) vs. U-500R (21.4 ± 6.7 min) and 169% increase in AUC_{GIR0–60min} (108.5 ± 22.0 mg/kg vs. U-500R (40.4 ± 10.0 mg/kg). These differences were all statistically significant. BIOD-531 was associated with an increased rate of absorption vs. HU 75/25 as measured by multiple PK parameters including a 66% shorter time to early ½ T_{max} (16.4 ± 4.9 min) vs. HU 75/25 (47.9 ± 2.6 min), an 18% shorter time to T_{max} (131.3 ± 43.4 min) vs. HU 75/25 (160.0 ± 11.9 min) and a 917% increase in early insulin exposure as measured by AUC_{ins0–30min} (1200 ± 141 min) vs. HU 75/25 (118 ± 22 mU*min/L). BIOD-531 was associated with a more rapid glucose lowering effect as measured by multiple PD parameters including a 59% faster time to onset of action (14.6 ± 6.0 min) vs. HU 75/25 (35.9 ± 7.9 min) and 375% increase in AUC_{GIR0–60min} (68.9 ± 13.4 mg/kg) vs. HU 75/25 (14.5 ± 4.7 mg/kg). With the exception of T_{max}, these differences were all statistically significant. The duration of action of BIOD-531 is approximately 18 hours, shorter than that of either comparator. All study drugs were well tolerated.

Conclusion: BIOD-531 is associated with more rapid onset of action than either marketed prandial/basal insulin product suggesting that it may provide improved mealtime glucose control with low injection volumes. Furthermore, the duration of action of BIOD-531 is consistent with a basal insulin.

Supported by: NIDDK Award R43DK096604

938

Pharmacokinetics and elimination of basal insulin peglispro (BIL) versus PEG-only in rats following a single IV or SC dose

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Background and aims: BIL (LY2605541) is a novel, 20-kDa PEGylated insulin lispro that has a large hydrodynamic size. It has a prolonged duration of action which is related to a delay in insulin absorption and a reduction in clearance. The aim of this study was to examine the pharmacokinetics and elimination of BIL compared with 20-kDa PEG only in rats.

Materials and methods: Male Sprague Dawley rats were given a 0.5-mg/kg IV or 2-mg/kg SC dose of BIL with ¹⁴C-labeled 20-kDa PEG portions of BIL or ¹²⁵I-labeled lispro portions of BIL. PEG only (¹⁴C- 20-kDa PEG) was dosed at 10 mg/kg IV or SC. Blood for serum, urine, and feces were collected up to 336 h postdose. Samples were analyzed for ¹⁴C using liquid scintillation counting, for ¹²⁵I using gamma counting, for BIL using ELISA, and for PEG-related products using liquid chromatography/mass spectrometry (MS) or size-exclusion chromatography.

Results: AUCs for BIL were 41% and 16% of circulating ¹⁴C after IV and SC dosing, respectively. AUCs for BIL were 63% and 31% of circulating ¹²⁵I for IV and SC dosing, respectively. After SC dosing, t_{1/2} of ¹⁴C was 4.4-fold longer than that of ¹²⁵I. After IV dosing, BIL Cl was 2.4-fold faster than that of ¹⁴C radioequivalents and 1.6-fold faster than that of ¹²⁵I radioequivalents indicating catabolism of BIL into products with longer half-lives. The t_{1/2} and Cl of ¹⁴C after PEG-only dosing were similar to those after BIL dosing. SC bioavailability of radiolabeled BIL was 23%–29%; absorption was ≥75%. The bioavailability of 20-kDa PEG-only was 78%. Excretion of radioactivity after IV or SC dosing of ¹⁴C-BIL was generally similar. In the BIL study, ¹⁴C was eliminated in urine and feces (urine:feces ratio, 0.9 for IV and 1.35 for SC). In the PEG-only study, most of the ¹⁴C was recovered in urine after IV or SC administration. In the BIL study, ≥86% of ¹²⁵I was eliminated in urine mainly as small peptides, amino acids or free ¹²⁵I. After SC ¹⁴C-BIL dosing, a mean ±SD of 2.5% ± 0.66% of the dose was excreted intact in urine. Based on qualitative MS analyses, the main PEG-related product found in serum and urine after BIL dosing was 20-kDa PEG with lysine attached. After PEG-only dosing, the main urinary product appeared to be intact PEG based on size-exclusion chromatography.

Conclusion: These data suggest that elimination of BIL is via catabolism to smaller peptides and/or amino acids and PEG with lysine attached. BIL is cleared faster than the products from catabolism. After BIL dosing, the PEG portion is eliminated via biliary and renal routes; after PEG alone, most of the ¹⁴C dose is eliminated renally. The PEG in BIL would only partially depend on renal function for elimination, unlike PEG-only. The protein attached to PEG may direct the elimination of PEG.

Supported by: Eli Lilly and Company

PS 075 Insulin treatment in the „real world“

939

Pump use is less frequent in minority youth: transatlantic analysis in three large registries representing Austria, Germany, England, Wales and the United States

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Background and aims: Use of insulin pumps has recently increased among children and adolescents with type-1 diabetes in many parts of the world. Patient and family preferences, beliefs and policies of healthcare professionals, expectations on metabolic goals, but also financial aspects / reimbursement by health insurance interact on the decision for or against CSII in individual patients. Each of these components depends on society and may be different for minority youth. Ideally, important treatment choices should be independent from the socio-economic background of a patient. The aim of this study was to evaluate this claim based on data from 3 large, multicenter registries.

Materials and methods: In total, 54,767 children and adolescents (<18 years) with T1D from the United States (T1D Exchange registry, n=13,966), England and Wales (National Paediatric Diabetes Audit, n=14,539) and Austria / Germany (DPV registry, n=26,262) with documented insulin therapy were available for analysis. Minority status was defined by ethnicity for UK and US, and by country of birth for Austria/Germany. Using a multivariable logistic regression model, the frequency of CSII was adjusted for differences in age, gender and diabetes duration among the 3 registries (SAS 9.4).

Results: Pump use differed among the 3 registries: 47.0 % in the US, 40.1 % in Austria/Germany and 13.9 % in the UK (p<0.001). After demographic adjustment, respective figures were 45.7 %, 40.5 % and 11.7 %. Despite different definitions, the percentage of minority youth was rather similar: 22.2 % in the US, 20.0 % in Austria/Germany and 23.8 % in the UK. In the whole cohort, 22.4 % of minority children, compared to 34.7 % of non-minority children, used insulin pumps (OR 0.543 [0.517, 0.570, p<0.001]). This difference was detected in all 3 registries: US: 29.3 % versus 50.6 % on CSII, OR 0.403 [0.369 - 0.441]; Austria/Germany: 30.9 % versus 41.9 %, OR 0.621 [0.580 - 0.664] and UK: 8.1 versus 14.8 % on CSII, OR 0.507 [0.446-0.577].

Conclusion: Even after taking differences among age, gender and duration of diabetes among the registries into account, the use of CSII in children and adolescents differs markedly between the US, central Europe and the UK, 3 wealthy regions of the world. However, in all 3 registries, minority youth are significantly less likely to be treated with CSII. The reasons for this are likely to be complex, including health beliefs/acceptance of technology, patient selection by treatment facilities, including potential language barriers relevant for (technical) patient education, which is more demanding for CSII therapy. In addition, financial aspects, such as differences in availability of health insurance or reimbursement of CSII therapy, may play a role. In order to provide access to modern diabetes therapy irrespective of social background, more research into barriers to CSII use for minority youth in different societies is required.

Supported by: Competence net Diabetes mellitus; HQIP; Helmsley trust

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Cardiovascular outcomes and their relationship to risk factors and baseline disease over 4 years when starting insulin in type 2 diabetes: the CREDIT study

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Background and aims: To evaluate the relationship between risk factors/disease at baseline and adverse cardiovascular (CV) outcomes over 4 years from starting insulin therapy in people with type 2 diabetes (T2DM) in routine clinical practice in Europe, Canada, and Japan.

Materials and methods: CREDIT (Cardiovascular Risk Evaluation in people with Type 2 Diabetes on Insulin Therapy) was a noninterventional study of people with type 2 diabetes beginning insulin therapy in Europe, North America, and Asia. Data from people with T2DM starting any insulin were collected in 12 countries over 4 years (\pm 6 months). Candidate explanatory variable for MACE+ (defined in Table) were age, sex, BMI, previous diagnosis of high blood pressure (BP), family history of premature CV disease, level of physical activity, smoking status, macrovascular disease, microvascular disease, HbA_{1c} when starting insulin, and number of noninsulin antidiabetic treatments, first insulin regimen, time from diagnosis of T2DM, insulin dose (U/kg), systolic BP, diastolic BP, heart rate, antihypertensive treatment, antiplatelet/anticoagulant treatment, and statin/fibrate treatment. These variables were conditioned for multivariable survival analyses using a stepwise reduction process with forced inclusion of HbA_{1c}. This enabled the estimate of the relationship between the variables and time to a composite of blindly adjudicated CV events.

Results: When starting insulin, participant characteristics (51% male) included median age of 61 years, 9.3 % HbA_{1c} (78 mmol/mol), and 9.0 years' duration of diabetes. Survival models were based on 286 events in 2974 people. First insulin regimen and starting HbA_{1c} were not predictive factors. The final multivariable Cox model included, as independent factors for MACE+, male gender, longer duration of diabetes, prior or current macrovascular disease, use of antihypertensive or of antiplatelet/anticoagulant medications, and lack of physical activity (Table).

Conclusion: Strong relationships were found between baseline factors and CV outcomes in people with T2DM starting insulin, after accounting for a number of explanatory characteristics. Evidence of CV disease at start of therapy (identified as prior macrovascular disease or use of antihypertensive/antiplatelet/anticoagulation therapy) was strongly predictive of a MACE+ event. Being physically active or being female was shown to have a strong protective effect for MACE+.

Factor	Modelled outcome	
	MACE+	
	HR (95% CI)	P Value
≥ 1 prior macrovascular disease (Y/N)	2.88 (2.21, 3.77)	< 0.0001
Gender (Female vs male)	0.60 (0.47, 0.77)	< 0.0001
Physical activity (yes vs no)	0.73 (0.58, 0.93)	0.0101
Duration of diabetes (years)	1.02 (1.00, 1.03)	0.0105
≥ 1 Blood pressure-lowering drug	1.65 (1.14, 2.39)	0.0076
Antiplatelet/anticoagulant therapy	1.32 (1.01, 1.72)	0.0436
MACE+ = MACE (CV death, MI, or stroke) or revascularisation, amputation, or hospitalisation for severe angina or heart failure. HR, hazard ratio; CI, confidence intervals; CV, cardiovascular, MI, myocardial infarction. Factors are as at baseline.		

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Adherence to insulin treatment in insulin naive type 2 diabetic patients: results of telephonic intervention

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Background and aims: The aim of the present study was to assess the efficacy of telephone support on insulin treatment adherence in insulin naive type 2 diabetic patients using different insulin treatment regimens (basal, basal-bolus and premix) in third care medical centers in Turkey

Materials and methods: In this 12 week-open label randomized multicenter study A total of 1456 insulin naive type2 diabetic patients (mean age 56±12yrs, diabetes duration 6,0±6 yrs, 49.1% female) were recruited in 14 third care endocrine centers from different city's in Turkey. Patients randomized to telephonic intervention (TI) (50.3%) and control group (C) (%49.7). Primary outcome was medication adherence.

Results: At the end of the 12th week 83.2 % of TI group and %70.3 of the Control group were adherent to treatment ($p<0.0001$). According to insulin treatment regimens adherence rates were %76.9,%78.6,%74.1 for Basal-Bolus (BB), Premix and Basal regimens respectively and insulin regimens were not different in TI group. While patients on premixed insulin regimen were more compliant to insulin treatment compared to other regimens in control group ($p<0.001$). Non adherent subjects had shorter duration of diabetes and higher HbA1c levels compared to adherent subjects in the whole study group ($p<0.01$). Multivariate analysis indicate that illiterates, unwillingness to therapy are the independent risk factors for decreased adherence to insulin therapy. Noncompliance rates for daily insulin injections were 27%,15%,15.8% for basal-bolus ,premixed and basal insulin regimens respectively and was higher in basal bolus group compare to other insulin regimens ($p<0.0001$). Reported dropout causes of insulin therapy were depend on patients (56.8%) ,doctors (24.8%) and adverse effects of insulin (4.8%). Frequency of hypoglycemia (minor and major) were 30.5%, 34%, 11.2% for Basal-Bolus, Premixed and Basal regimens respectively ($p<0.0001$).

Conclusion: Telephonic intervention increases adherence and compliance to insulin treatment in insulin naive type 2 diabetic patients. Telephone communication between diabetes management team and diabetic patients could be considered as a part of routine follow up procedure in diabetes treatment

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Trajectories of HbA1c after initiation of insulin therapy in type 2 diabetes mellitus patients

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Background and aims: ADA/EASD guidelines for the management of type 2 diabetes mellitus (T2DM) recommend HbA1c levels of ≤ 53 mmol/mol. About 25% of all T2DM patients initiate insulin to reach this target. However, trajectories of the HbA1c levels after initiation of insulin have never been described and not much is known about characteristics associated with reaching and sustaining the HbA1c target. The aim of the current study was to identify subgroups of T2DM patients with distinct HbA1c trajectories after the start of insulin treatment and factors associated with reaching and sustaining the target HbA1c level (≤ 53 mmol/mol).

Materials and methods: 798 T2DM patients from the Diabetes Care System (DCS) cohort of 9849 T2DM patients were included. Inclusion criteria: aged ≥ 40 year, initiated insulin during follow up after reaching a HbA1c level of ≥ 53 mmol/mol, with a follow up ≥ 2 years. Latent Class Growth Modelling (LCGM) was used to identify trajectories of HbA1c after the initiation of insulin. Cox regression analyses were used to determine which characteristics were associated with reaching and sustaining target HbA1c ≤ 53 mmol/mol.

Results: Four classes of HbA1c development were identified (figure 1). In the first class (3.8%) an gradual increase in HbA1c over time was seen, the second class (3.3%) showed a decrease in HbA1c during follow-up. The third class (5.1%) showed a good response to insulin treatment. The fourth class (87.8%) consisted of T2DM patients with stable HbA1c levels around 58 mmol/mol. During a mean follow-up of 4.5 years (SD 3.0), 174 patients (21.8%) reached and sustained the HbA1c target of ≤ 53 mmol/mol. Higher HbA1c levels at time of insulin initiation (HR 0.80 (95% CI 0.68 to 0.92)) and advanced age at baseline (HR 1.03 (95% CI 1.02 to 1.05)) were associated with reaching and sustaining the HbA1c target of ≤ 53 mmol/mol.

Conclusion: Less than a quarter of the patients achieved and sustained HbA1c levels ≤ 53 mmol/mol after insulin initiation. Low HbA1c level at the start of insulin, and higher age were associated with reaching the HbA1c target. Initiating insulin earlier, i.e. at lower HbA1c levels in T2DM patients, may improve the likelihood of achieving and sustaining glycemic control.

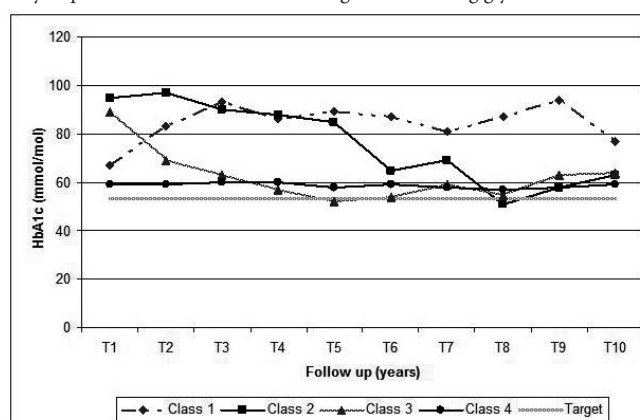


Figure 1 Trajectories of HbA1c over ten years in four classes of T2DM patients after initiation of insulin.

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Four-year analysis of insulin dose and hypoglycaemia in the real-world treatment of type 2 diabetes: results from the CREDIT study

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Background and aims: CREDIT (Cardiovascular Risk Evaluation in People with Type 2 Diabetes on Insulin Therapy) was a noninterventional study of people starting insulin therapy in routine clinical practice in Europe, N. America, and Asia and followed for 4 years (n=2272 with 4-yr data).

Materials and methods: Baseline data were collected retrospectively to avoid influence on choice of insulin; data were extracted from regular clinical records. For this analysis, records of symptomatic and severe hypoglycaemia were extracted for the 6 months prior to the 1, 2, 3, and 4-year follow-up intervals.

Results: Total daily insulin dose increased 25.6% from year 1 to year 4 for all insulin regimens as taken at times of follow-up (Table). Overall, the percentage of patients reporting symptomatic hypoglycaemia was 18.5% at year 1 and 16.6% at year 4; event rates were between 0.8 and 1.5 per person across years and insulin regimens. Prevalence of severe hypoglycaemia was $< 3.5\%$ for all insulins and years (except mealtime insulin alone at year 4); event rates ranged from 0.0 to 0.2 per person.

Conclusion: These CREDIT study results show that regardless of insulin regimen utilised (whether maintained from start or changed), total daily insulin dose increases over time, while symptomatic and severe hypoglycaemia were

relatively unchanged. Increased hypoglycaemia on insulin does not appear to be a necessary consequence of increased need for exogenous insulin with time in routine care.

	Basal alone	Basal + mealtime	Mealtime alone	Premix alone	All ^a
Year 1	n=1131	n=571	n=88	n=735	n=2734
Year 2	n= 957	n=667	n=65	n=677	n=2581
Year 3	n= 815	n=722	n=59	n=606	n=2435
Year 4	n= 671	n=741	n=54	n=572	n=2272
Total insulin dose by actual regimen taken (U/kg/day), mean (SD)					
Year 1	0.32 (0.19)	0.60 (0.25)	0.30 (0.19)	0.45 (0.23)	0.43 (0.25)
Year 2	0.35 (0.20)	0.64 (0.28)	0.27 (0.17)	0.49 (0.27)	0.47 (0.27)
Year 3	0.37 (0.22)	0.67 (0.32)	0.28 (0.15)	0.52 (0.29)	0.51 (0.30)
Year 4	0.38 (0.22)	0.69 (0.30)	0.33 (0.33)	0.54 (0.31)	0.54 (0.31)
≥1 Confirmed symptomatic hypoglycaemia in last 6 months, n (%)					
Confirmed symptomatic hypoglycaemia in last 6 months (events/person), mean (min, max)					
Year 1	170 (15.0) 0.8 (0, 75)	122 (21.4) 1.4 (0, 126)	17 (19.3) 0.6 (0, 10)	156 (21.3) 1.1 (0, 60)	504 (18.5) 1.1 (0, 126)
Year 2	148 (15.5) 1.0 (0, 70)	141 (21.2) 1.2 (0, 130)	13 (20.0) 1.1 (0, 28)	138 (20.4) 1.1 (0, 53)	471 (18.3) 1.0 (0, 130)
Year 3	108 (13.3) 0.8 (0, 30)	133 (18.5) 1.0 (0, 52)	10 (16.9) 0.4 (0, 4)	113 (18.7) 0.9 (0, 45)	390 (16.1) 0.9 (0, 52)
Year 4	100 (14.9) 1.2 (0, 45)	138 (18.6) 1.0 (0, 48)	8 (14.8) 0.9 (0, 30)	109 (19.1) 1.0 (0, 34)	376 (16.6) 1.0 (0, 48)
≥1 Documented severe hypoglycaemia in last 6 months, n (%)					
Severe hypoglycaemia in last 6 months (events/person), mean (min, max)					
Year 1	23 (2.0) 0.0 (0, 3)	16 (2.8) 0.1 (0, 15)	0 (0.0) 0.0 (0, 0)	10 (1.4) 0.0 (0, 7)	51 (1.9) 0.0 (0, 15)
Year 2	25 (2.6) 0.1 (0, 5)	14 (2.1) 0.0 (0, 3)	1 (1.5) 0.0 (0, 1)	3 (0.4) 0.0 (0, 1)	44 (1.7) 0.0 (0, 8)
Year 3	27 (3.3) 0.1 (0, 10)	17 (2.4) 0.1 (0, 12)	1 (1.7) 0.0 (0, 2)	4 (0.7) 0.0 (0, 5)	49 (2.0) 0.1 (0, 12)
Year 4	22 (3.3) 0.2 (0, 12)	19 (2.6) 0.1 (0, 20)	3 (5.6) 0.2 (0, 10)	2 (0.3) 0.0 (0, 1)	46 (2.0) 0.1 (0, 20)

^a "All" includes those categorised as "Other" or "No insulin" (data not shown)

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Insulin treatment across Europe: results from the GUIDANCE study
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Background and aims: Given the increasing prevalence of type 2 diabetes (T2DM), insulin treatment will be needed for a growing number of patients. Traditionally the conversion to insulin therapy has been managed in the secondary care setting, but more and more primary care physicians are involved in insulin therapy. We compared insulin treated patients across eight European countries, stratified to treatment setting.

Materials and methods: Data were available from the medical records of 1960 insulin treated T2DM patients of eight European countries, from mainly primary care (Belgium, France, Germany, Sweden, the Netherlands) and mainly secondary care (Italy, Ireland, the United Kingdom) countries. Patients from 'mainly primary care' and 'mainly secondary care' countries were compared for patient variables: age, sex, duration of diabetes, HbA1c, BMI, micro- and macro vascular complications, type of insulin treatment; and service variables: access of a nurse or a dietician, physician's number of T2DM patients treated, and physician's feelings about the diabetes guidelines. MLwiN for binomial multilevel analysis was used to analyse the relation of the patient and service variables with insulin treated patients from mainly primary and secondary care countries.

Results: Type of insulin treatment differed across European countries; insulin treated patients from mainly secondary care countries used more often short acting analogues (OR 2.54, 95%CI 1.89-3.43) and less often either NPH (OR 0.45, 95%CI 0.28-0.73), long acting analogues (OR 0.50, 95%CI 0.36-0.68) or short acting human insulin (OR 0.59, 95%CI 0.37-0.92) compared to those in mainly primary care countries. Further, insulin treated patients from the mainly secondary care countries showed higher HbA1c levels (OR 1.49, 95%CI 1.34-1.66) and had more often access to a dietician (OR 2.54, 95%CI 1.89-3.43), but were less likely to have macro vascular complications (OR 0.65, 95%CI 0.49-0.86). No clinical relevant differences were found for the other variables.

Conclusion: The results of this large European cross-sectional study show that T2DM patients on insulin and mostly treated in a primary care setting in five European countries did not differ from the same category of patients mainly treated in outpatient clinics or hospitals in three other countries, with the exception of type of insulin, HbA1c and the presence of macro vascular

complications. The decision to treat a T2DM patient in primary or secondary care is likely not based on clinical arguments.

	Belgium	France	Germany	Italy	Ireland	Sweden	Netherlands	UK
Patients on insulin therapy n (% of total)	197 (19)	175 (17)	362 (38)	380 (39)	188 (20)	197 (36)	166 (16)	295 (29)
Type of insulin treatment NPH								
Long acting analogues	15.6	9.7	20.3	8.3	2.6	0.5	10.2	9.3
Short acting analogues	29.6	79.0	43.7	71.1	63.5	76.2	59.6	51.7
Short acting human insulin	18.1	20.5	23.6	63.8	30.2	28.2	23.5	62.3
Age (SD)	69.8 (10.4)	67.5 (10.1)	68.7 (8.7)	69.3 (8.5)	65.5 (10.0)	67.5 (10.1)	67.2 (10.3)	62.1 (10.4)
Male (%)	44.7	59.4	46.4	52.9	60.6	60.4	57.8	57.6
Duration of T2DM	13.9 (8.5)	15.5 (9.7)	13.7 (8.0)	17.3 (8.6)	10.6 (7.2)	14.3 (8.4)	9.3 (4.6)	12.3 (7.1)
HbA1c (%)	7.5 (1.1)	7.6 (1.2)	7.7 (1.1)	8.1 (1.4)	8.3 (1.9)	7.7 (1.2)	7.4 (0.9)	8.4 (1.6)
BMI (kg/m ²)	29.9 (5.3)	30.4 (6.0)	31.9 (5.7)	29.0 (5.1)	32.8 (7.1)	30.1 (4.9)	30.2 (5.8)	34.0 (6.8)
Micro vascular complications (% yes)	35.7	28.4	37.1	43.0	20.1	39.8	14.5	36.7
Macro vascular complications (% yes)	47.2	36.4	51.4	38.8	32.3	29.6	27.7	23.0
Setting (% primary care)	95	92	79	4	16	100	100	48
Practice nurse (% with access)	93	100	81	89	96	100	100	100
Dietitian (% with access)	100	98	61	90	97	100	100	100
Number of T2DM patients	18 (17)	17 (29)	65 (67)	55 (34)	52 (36)	4 (2)	60 (109)	29 (21)
Feelings about guidelines by physician (0-100%)	62 (13)	66 (9)	58 (9)	70 (7)	67 (10)	64 (7)	73 (3)	62 (7)

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Metabolic control in type 2 diabetic patients with conventional or intensified insulin therapy: a retrospective single centre survey over a period of 22 years

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Background and aims: The 2012 EASD/ADA position statement on management of hyperglycaemia in type 2 diabetes recommends mealtime insulin, consisting of one to three injections, when basal insulin has been titrated to an acceptable fasting glucose but HbA1c remains above target. The progression from basal insulin to a twice daily pre-mixed insulin (CT) is regarded as a "less studied alternative". Multiple insulin injection therapy (MIT) is regarded to be more easily adaptable to the patients needs. Therefore it is supposed that MIT is associated with better metabolic control and less hypoglycaemia compared to CT in patients with diabetes type 2 (DM2).

Materials and methods: HbA1c, insulin dose, body weight and incidence of non severe and severe hypoglycaemia were analysed in respect to the strategy of insulin therapy applied (MIT or CT). 20943 visits of 1447 insulin treated people with DM2 in an university outpatient department for endocrinology and metabolic diseases from a period of 22 years (9.1.1990 to 20.9.2012) were included. Data were drawn from the electronic patient record EMIL®. Because the strategy of insulin therapy changed over the long period, we compared visits with CT and visits with MIT. All visits with one or two insulin injections were classified as CT, visits with more than two injections as MIT. Visits with basal insulin only were not included. Non severe hypoglycaemia was defined as a condition with typical symptoms (e.g., sweating, feeling shaky, impaired concentration) which disappeared quickly after carbohydrate ingestion or a plasma glucose < 2.7 mmol/l with or without typical symptoms. Severe hypoglycaemia was defined as necessity of intravenous glucose injection. All patients participated in a structured education programme at least once. The normal range and mean value of HbA1c changed from 1999 to 2012, therefore we adjusted HbA1c according to the mean normal value of healthy people to the DCC trial (5.05 %).

Results: MIT was used in 13901 (66.4 %) of all 20943 visits. Compared to CT these patients were younger (62.0 ± 10.1 vs. 68.7 ± 9.9 years; p < 0.0001), time since diabetes diagnosis was longer (16.5 ± 8.7 vs. 15.8 ± 8.1 years; p < 0.0001), had higher BMI (32.8 ± 6.1 vs. 30.9 ± 5.3 kg/m²; p < 0.0001), a higher daily insulin dose (76.4 ± 52.0 vs. 46.5 ± 26.9 IU/day; p < 0.0001), and lower HbA1c (7.7 ± 1.4 vs. 7.9 ± 1.4 %; p < 0.0001). In MIT more non severe hypoglycaemic incidences per week (0.3 ± 0.7 vs. 0.2 ± 0.8; p = 0.01) occurred, episodes of severe hypoglycaemia/last 12 months were rare and comparable (0.02/Patient/year) with both insulin therapy strategies. Multiple, linear regression analysis showed that the only parameter associated with HbA1c was the insulin dose (β = 0.222, p < 0.001). Higher HbA1c is weakly associated with higher daily insulin dose, but not age (β = -0.019, p = 0.471), time since diabetes diagnosis (β = 0.060, p = 0.023) as well as incidence of non severe hypoglycaemia/week (β = 0.006, p = 0.797).

Conclusion: MIT and CT insulin therapy result in comparable metabolic control with HbA1c below 8 %. MIT is associated with higher BMI and

higher incidence of non severe hypoglycemia. People with DM2 should be actively involved in the decision which insulin therapy to choose considering the life style and convenience. Therapy strategies can be swapped if necessary.

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Less nocturnal hypoglycaemia and weight gain with new insulin glargine 300 U/ml vs 100 U/ml: 1-year results in people with type 2 diabetes using basal insulin and OADs (EDITION 2)

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Background and aims: EDITION 2 investigated glycaemic control and hypoglycaemia using new insulin glargine 300 U/ml (Gla-300) versus glargine 100 U/ml (Gla-100) in people with type 2 diabetes mellitus on basal insulin plus OAD(s).

Materials and methods: In EDITION 2, 811 adults with type 2 diabetes mellitus and inadequate control of HbA_{1c} were randomised to receive either Gla-300 or Gla-100 for 6 months. In this 6-month open-label extension, participants continued to receive Gla-300 or Gla-100 once daily plus OADs; 315 (78%) participants in the Gla-300 group and 314 (77%) participants in the Gla-100 group completed 12 months of treatment.

Results: Improved control of HbA_{1c} was maintained at 12 months with each regimen. Over 12 months, per participant-year event rates of confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe nocturnal hypoglycaemia were 37% lower with Gla-300 than Gla-100 (1.74 vs 2.77, RR: 0.63 [95% CI: 0.42 to 0.96]). Fewer participants experienced ≥ 1 confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe nocturnal hypoglycaemic event with Gla-300 than Gla-100 (RR: 0.84 [95% CI: 0.71 to 0.99]). Severe hypoglycaemia at any time of day was infrequent, experienced by 7 participants in the Gla-300 group and 6 participants in the Gla-100 group. Body weight increase was observed in both groups, and was significantly less with Gla-300 than Gla-100 (LS mean [95% CI]: 0.42 [0.04 to 0.80] versus 1.14 [0.76 to 1.52] kg, $p=0.0091$). No between-treatment differences in adverse events were seen.

Conclusion: Over 12 months of treatment, people with type 2 diabetes mellitus using Gla-300 and OADs had comparable glycaemic control, and experienced fewer nocturnal hypoglycaemic events and less weight gain compared with those using Gla-100.

Table – Glycaemic control and hypoglycaemia over 12 months in the EDITION 2 study

		HbA _{1c} (%)	
mITT population		Gla-300 (N=403)	Gla-100 (N=406)
Baseline	Mean (SD)	8.26 (0.86)	8.21 (0.77)
Change from baseline to month 12 (OC)	Mean (95% CI)	-0.55 (-0.67 to -0.44)	-0.50 (-0.61 to -0.39)
	LS mean difference (95% CI)	0.06 (-0.22 to 0.10)	
		Nocturnal hypoglycaemia (00:00–05:59 h)	
		Gla-300 (N=403)	Gla-100 (N=406)
Any hypoglycaemia		Hypoglycaemia at any time of day (24 h)	
		Gla-300 (N=403)	Gla-100 (N=406)
Baseline to month 12	% people ≥ 1 event	39.7	46.1
	RR ^a (95% CI)	0.86 (0.73 to 1.01)	0.96 (0.90 to 1.03)
	Events/participant-year	1.80	2.94
	RR ^a (95% CI)	0.61 (0.41 to 0.92)	0.87 (0.70 to 1.07)
Confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe hypoglycaemia			
Baseline to month 12	% people ≥ 1 event	37.5	44.6
	RR ^a (95% CI)	0.84 (0.71 to 0.99)	0.96 (0.89 to 1.02)
	Events/participant-year	1.74	2.77
	RR ^a (95% CI)	0.63 (0.42 to 0.96)	0.88 (0.71 to 1.09)

CI, confidence interval; mITT, modified intention-to-treat; OC, observed case; RR^a, relative risk; RR^b, rate ratio

Clinical Trial Registration Number: NCT01499095

Supported by: Sanofi

PS 076 Glucose variability in insulin treatment

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New insulin glargine 300 U/ml: glycaemic control and hypoglycaemia in insulin-naïve people with type 2 diabetes mellitus (EDITION 3)

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Background and aims: EDITION 3 studied the efficacy and safety of new insulin glargine 300 U/ml (Gla-300) vs glargine 100 U/ml (Gla-100) in people with type 2 diabetes mellitus uncontrolled on non-insulin therapy.

Materials and methods: In this 6-month, multicentre, open-label study, participants were randomised to once-daily Gla-300 or Gla-100 in the evening while stopping sulfonylureas. Insulin was titrated seeking FPG 4.4–5.6 mmol/l. The primary endpoint was HbA_{1c} change to month 6. The main secondary endpoint was percentage of participants with ≥ 1 confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe nocturnal (00:00–05:59 h) hypoglycaemic event from week 9 to month 6.

Results: In total, 878 participants were randomised (baseline characteristics: BMI 33.0 kg/m²; diabetes duration 9.8 years; HbA_{1c} 8.5 % [70 mmol/mol]). HbA_{1c} decreased similarly with both treatments. LS mean changes (SE) were -1.42 [0.05] % (-15.5 [0.5] mmol/mol) with Gla-300 and -1.46 (0.05) % (-16.0 [0.5] mmol/mol) with Gla-100, and LS mean difference (95% CI) was 0.04 (-0.09 to 0.17) % (0.4 [-1.0 to 1.9] mmol/mol). The relative risk of experiencing ≥ 1 confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe nocturnal hypoglycaemic event with Gla-300 vs Gla-100 was 0.76 (95% CI: 0.59 to 0.99) for the 6-month treatment period, 0.74 (0.48 to 1.13) for baseline to week 8, and 0.89 (0.66 to 1.20) ($p=0.45$) for week 9 to month 6. Using a stricter threshold of <3.0 mmol/l (<54 mg/dl), relative risks of experiencing ≥ 1 confirmed or severe nocturnal hypoglycaemia were 0.59 (0.33 to 1.06) for the entire 6-month treatment period, 0.73 (0.30 to 1.79) for the first 8 weeks of treatment, and 0.61 (0.31 to 1.21) for week 9 to month 6. Rates (per participant-year) of confirmed (≤ 3.9 mmol/l) or severe events at any time of day (24 h) were lower with Gla-300 (rate ratio 0.75 [0.57 to 0.99]) over 6 months. Severe hypoglycaemia was infrequent (0.9% of participants experienced ≥ 1 event in both groups). Mean weight change was +0.4 (SD 3.8) kg with Gla-300 and +0.7 (3.8) kg with Gla-100. No differences in adverse events were seen.

Conclusion: In insulin-naïve people with type 2 diabetes mellitus, new insulin glargine 300 U/ml provides comparable effective glycaemic control, with less hypoglycaemia, compared with glargine 100 U/ml.

Table: Hypoglycaemia in the EDITION 3 study: 6-month study period (safety population)

		Nocturnal (00:00–05:59 h)		Any time of day (24 h)	
		Gla-300 (N=435)	Gla-100 (N=438)	Gla-300 (N=435)	Gla-100 (N=438)
Confirmed (≥ 3.9 mmol/l [≥ 70 mg/dl]) or severe	% people ≥ 1 event	17.9	23.5	46.2	52.5
	RR ^a (95% CI)	0.76 (0.59 to 0.99)		0.88 (0.77 to 1.01)	
	Events/participant-year	1.31	1.34	6.41	8.50
	RR ^a (95% CI)	0.98 (0.64 to 1.48)		0.75 (0.57 to 0.99)	
Confirmed (< 3.0 mmol/l [< 54 mg/dl]) or severe	% people ≥ 1 event	3.9	6.6	9.9	16.2
	RR ^a (95% CI)	0.59 (0.33 to 1.06)		0.61 (0.43 to 0.87)	
	Events/participant-year	0.11	0.19	0.40	0.54
	RR ^a (95% CI)	0.61 (0.33 to 1.15)		0.75 (0.47 to 1.22)	
Documented symptomatic (≤ 3.9 mmol/l [≤ 70 mg/dl])	% people ≥ 1 event	12.4	15.5	30.6	35.8
	RR ^a (95% CI)	0.80 (0.58 to 1.12)		0.85 (0.71 to 1.03)	
	Events/participant-year	0.76	0.84	2.33	3.76
	RR ^a (95% CI)	0.90 (0.55 to 1.48)		0.62 (0.44 to 0.87)	
Documented symptomatic (< 3.0 mmol/l [< 54 mg/dl])	% people ≥ 1 event	3.2	6.4	7.6	13.9
	RR ^a (95% CI)	0.51 (0.27 to 0.94)		0.55 (0.37 to 0.82)	
	Events/participant-year	0.10	0.18	0.24	0.45
	RR ^a (95% CI)	0.55 (0.28 to 1.07)		0.55 (0.35 to 0.85)	

CI, confidence interval; RR^a, relative risk; RR^b, rate ratio.

Clinical Trial Registration Number: NCT01676220

Supported by: Sanofi.

948

Similar efficacy and safety with LY2963016 insulin glargine compared with insulin glargine in patients with type 2 diabetes mellitus: the ELEMENT 2 study

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Background and aims: LY2963016 (LY IGLar) and insulin glargine (Sanofi-Aventis; IGLar) are both insulin glargine products, with identical amino acid sequences. Even with identical primary structure, protein-based therapeutics manufactured by distinct processes must be shown to be clinically similar.

Materials and methods: This was a 24-week, Phase 3, randomized, double-blind, parallel study to compare the efficacy and safety of LY IGLar and IGLar. The primary objective was to test the non-inferiority (0.3% margin) of LY IGLar to IGLar as measured by change in HbA_{1c} from baseline to 24 weeks in patients with T2DM on ≥ 2 oral antihyperglycaemic medications (OAMs). Testing for non-inferiority of IGLar to LY IGLar was also performed and pre-specified as a complementary hypothesis, which if met along with the primary aim, would demonstrate equivalent efficacy between LY IGLar and IGLar. Patients were insulin-naïve (HbA_{1c} $\geq 7.0\%$ to $\leq 11.0\%$) or previously on IGLar (HbA_{1c} $\leq 11.0\%$). Insulin-naïve patients started on 10 U/day while those previously on IGLar started at an equivalent dose to their prestudy IGLar dose. Both groups followed a patient-driven titration algorithm until fasting blood glucose reached ≤ 5.6 mmol/L. For blinding purposes, patients randomized to treatment were provided syringes and insulin vials contained in a partial cover that concealed distinguishing differences in vial appearance.

Results: Both treatment groups had within-group similarly significant ($p < .001$) decreases in mean HbA_{1c} values ($\sim -1.3\%$ [Endpoint: LY IGLar, 7.04%; IGLar, 6.99%]). Change in HbA_{1c} from baseline with LY IGLar was non-inferior to IGLar (Table). Noninferiority of IGLar to LY IGLar was also demonstrated; thus, criteria for equivalence in clinical efficacy between LY IGLar and IGLar were met. There were no treatment differences in secondary efficacy or safety outcomes, including hypoglycaemia and treatment-emergent antibody response, in the total population and in the insulin-naïve/prestudy IGLar subgroups. The treatment groups had similar mean (SD) overall nocturnal hypoglycaemia rate (events/patient/year) (LY IGLar: 7.46 (11.73),

IGlar: 8.08 (14.62), $p = .686$) and only 2 patients per group experienced severe hypoglycaemia. Adverse event frequencies (LY IGLar, 52%; IGLar, 48%; $p = .31$) were similar. No treatment differences were observed in mean (SD) weight change (kg) at endpoint, LY IGLar: 2.09 (3.80), IGLar: 2.33 (3.39), $p = .334$).

Conclusion: LY IGLar compared with IGLar in combination with OAMs provided equivalent efficacy and similar safety profiles in patients with T2DM.

Table

Outcome Measure	Total LY IGLar N=376 ^a	Total IGLar N=380 ^a	p-value	Insulin Naïve LY IGLar N=220 ^a	Insulin Naïve IGLAR N=235 ^a	p-value
HbA _{1c} (%)						
Baseline	8.350 (0.06)	8.310 (0.06)	.611	8.502 (0.07)	8.425 (0.07)	.449
Change from Baseline (LOCF)	-1.286 (0.06)	-1.338 (0.06)	.403	-1.475 (0.07)	-1.536 (0.07)	.432
LSM Diff [95% CI] (LOCF)	0.052 [-0.070, 0.175]			0.061 [-0.061, 0.214]		
N (%) of Patients Reaching HbA _{1c} $< 7.0\%$	160 (49)	197 (53)	.340	117 (54)	139 (60)	.355
FBG (mmol/L) by SMBG						
Baseline	8.8 (0.1)	8.9 (0.1)	.837	9.6 (0.2)	9.5 (0.2)	.528
Change from Baseline (LOCF)	-2.6 (0.2)	-2.6 (0.2)	.685	-3.2 (0.2)	-3.1 (0.2)	.726
LSM Diff [95% CI] (LOCF)	-0.07 [-0.40, 0.26]			-0.07 [-0.478, 0.334]		
Daily Mean Blood Glucose (LOCF), mmol/L	7.6 (0.1)	7.7 (0.1)	.401	7.4 (0.1)	7.4 (0.1)	.842
Insulin Dose, U/kg/day Mean (SD)	0.50 (0.03)	0.48 (0.03)	.393	0.45 (0.03)	0.46 (0.03)	.640
Total Hypoglycaemia ^b Rate (events/patient/year)	21.3 (24.4)	22.3 (28.2)	.995	21.6 (25.6)	22.9 (27.4)	.432
Mean (SD), Overall	14 (3.8)	14 (3.8)	>.999	10 (4.7)	13 (5.8)	.612

^aFull Analysis Set, N numbers reflect maximum sample size

^bIncluding events with blood glucose ≤ 3.9 mmol/L. If blood glucose was available

LOCF = last observation carried forward (endpoint); LSM = least squares mean; TEAR = treatment emergent antibody response (including patients who were antibody negative at baseline and developed antibody binding values $\geq 1.26\%$ postbaseline or patients with detectable antibody levels at baseline with at least a 1% increase in antibody binding value and which is 30% greater than baseline).

Clinical Trial Registration Number: NCT01421459

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949

Insulin glargine 300 U/ml vs 100 U/ml: glucose profiles of morning vs evening injections in adults with type 1 diabetes mellitus measured with continuous glucose monitoring (CGM)

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Background and aims: Previous euglycaemic clamp studies in type 1 diabetes (T1DM) showed that insulin glargine 300 U/ml (Gla-300) provides evenly distributed 24-h coverage due to low fluctuation and high reproducibility in insulin glargine exposure. The primary objective of this exploratory study in T1DM was to compare the percentage of time in the glycaemic range of 4.4–7.8 mmol/L, utilising CGM during Gla-300 vs insulin glargine 100 U/ml (Gla-100) treatment. HbA_{1c}, mean glucose level, glucose variability and incidence of hypoglycaemia were evaluated.

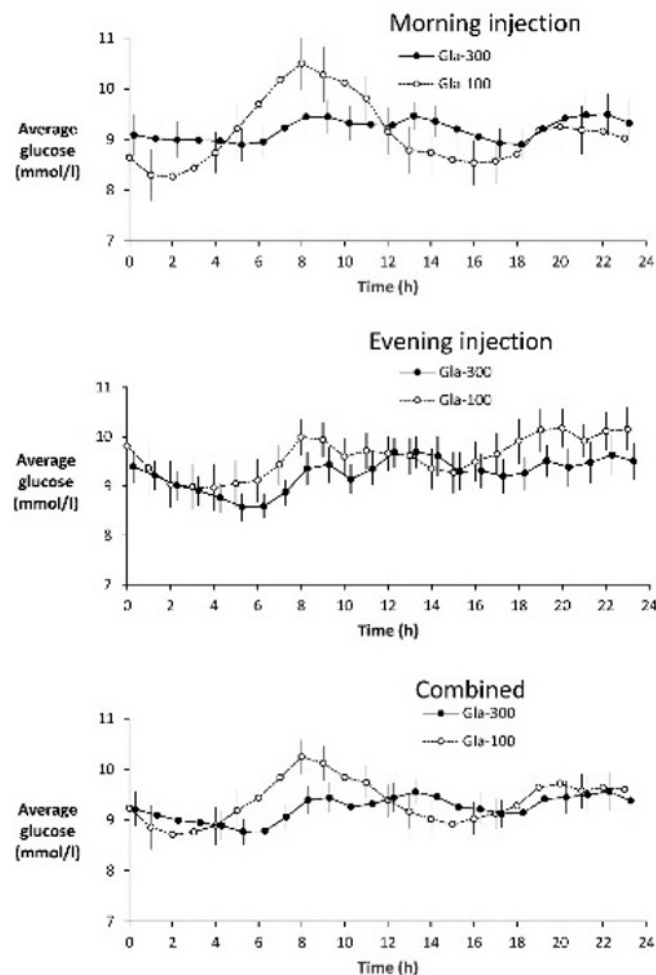
Materials and methods: In this multicentre, 16-week, open-label phase 2 study, 59 adults with T1DM (mean age 44.2 y; DM duration 22.1 y; baseline HbA_{1c} 7.46 %) were randomised (1:1) to Gla-300 or Gla-100 once daily, with crossover between morning and evening injections. CGM data were evaluated during the last 2 weeks of each 8-week study period.

Results: HbA_{1c} was similar for Gla-300 and Gla-100 (7.02 % vs 7.17 %) at week 16, and mean glucose (CGM sensor glucose [SG] from week 15–16) was similar for Gla-300 vs Gla-100 (9.2 mmol/L vs 9.4 mmol/L). The percentage of time SG was within the target range 4.4–7.8 mmol/L with Gla-300 and Gla-100 was 32% vs 31% (LS mean, difference [95% CI] 0.75 [−3.61 to 5.12] %). Hypoglycaemia was lower for subjects receiving Gla-300 vs Gla-100 using SG thresholds of 3.9, 3.3, 2.8, and 2.2 mmol/L. Hyperglycaemia above thresholds of 10.0, 13.9, and 22.2 mmol/L was also lower. Frequency (%) of SG values 13.9 mmol/L for people on Gla-300 vs Gla-100 was mean (SD) 4.7 (3.3) vs 5.4 (4.3) and 12.7 (8.0) vs 16.4 (10.5), respectively. Mean 24-h glucose profiles, averaging SG during the last 2 weeks of each treatment period for all subjects on Gla-300 or Gla-100, showed less excursion on Gla-300 (Figure). When morning and evening injection periods were combined, all metrics for within-subject glycaemic variability were consistently lower with Gla-300: total standard deviation was lower by 7.4%, within day variability was lower by 5.4%, variability between daily means was lower by 14.3% and variability between days for the same time of day was lower by 7.2%.

Conclusion: In 59 adults with T1DM, Gla-300 injected either in the morning or the evening provided similar overall glucose control and percentage time

in target SG range (4.4–7.8 mmol/l) vs Gla-100, with less hypoglycaemia, a mean glucose profile for all subjects with less glucose excursion, and lower within- and between-day glucose variability.

SG profiles (mean [SE], mmol/l) during the last 2 weeks of each treatment period by time of day



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Getting the time right: is there any relationship between insulin administration-to-meal ingestion time interval and blood glucose control in type 2 diabetes?

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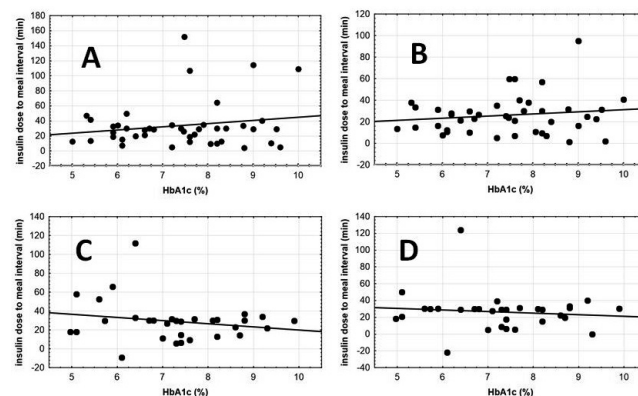
Background and aims: Patients treated with short-acting or premixed analog insulins are advised to take them immediately before a meal while those treated with classic human insulin are instructed to take insulin 20–30 minutes prior to a meal. The patients find the latter piece of advice difficult to comply with, and whether the compliance with recommended insulin dose-to-meal time interval (I-to-M, in minutes) has any relevance to metabolic control is unknown. We conducted a prospective observational study aiming at assessing the relationship between glucose control and mean duration of I-to-M.

Materials and methods: The study group consisted of 90 type 2 diabetes patients (mean age 64 ± 12 years, duration of diabetes 9.4 ± 4.7 years, HbA1c $7.5 \pm 1.5\%$, BMI 30.8 ± 4.4 kg/m²), treated with insulin twice daily (before breakfast and evening meal), who were asked to record the actual hours of insulin injections and meal initiation for 30 consecutive days for I-to-M to be calculated. HbA1c was measured within 10 days after recordings were completed.

Results: There was no correlation between HbA1c and mean I-to-M in the whole study group. However, in the patients treated with analog insulins ($n=51$) there was a trend to positive correlation between HbA1c and mean I-to-M, while in those treated with human insulins ($n=39$) the trend was opposite (Fig. 1).

Conclusion: We conclude that compliance with the recommended pre-meal insulin dose timing might have some, albeit minor, impact on overall glucose control in type 2 diabetes.

Figure 1. Relationship between HbA1c and mean insulin dose to meal time interval in type 2 diabetes patients taking analog insulin in the morning (A) and evening (B) or taking human insulin in the morning (C) and evening (D).



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The impact of pure protein load on glucose levels in type 1 diabetes patients treated with insulin pumps

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Background and aims: It is well recognized that the mealtime insulin requirement in type 1 diabetes (T1DM) patients is driven mostly by carbohydrate content and controlling it may improve glucose levels. However, it is uncertain whether the calculation of other food components may optimize glycemic control. The aim of this study was to estimate the impact of pure protein load on glucose levels in T1DM patients treated with personal insulin pumps.

Materials and methods: We examined 10 T1DM patients (6 females, 4 males, mean age - 32.3 years, mean T1DM duration - 11.7 years, mean HbA1c - 6.85%) treated with insulin pump (Medtronic Paradigm 722 or Veo) equipped with a continuous glucose monitoring system (CGMS) option. In phase I of the study, baseline insulin infusion was optimized to minimize differences in fasting glucose levels to less than 30 mg/dL between any two time points between 9 am and 3 pm. The procedure of optimization was based on a retrospective analysis of CGMS records and included increasing or decreasing the rate of basal insulin infusion two hours before the observed rise or fall in glucose level. Then, the new settings of the basal infusion rate were rechecked with CGMS to meet the study criteria. In phase II, the patients were exposed to single, pure protein load (Protifar, Nutricia) at the dose of 0.3 g/kg (0.34 mL of Protifar/kg dissolved in 200 mL of water) of body weight administered at 9 a.m. Such a dose of protein is an equivalent of the usual protein portion in a medium-size meal, based on the dietary recommendations for patients with diabetes (15–20% of energy). The rate of basal insulin infusion during protein load was the same as defined initially in phase I, no modifications or extra insulin boluses were permitted. CGMS record was performed in both phases, during which the patients avoided physical activity. Glucose patterns were defined during 6 hours of phase I (fasting) and phase II (protein load).

Results: Mean baseline glucose level was 119.8 and 117.6 mg/dL for Phase I and Phase II, respectively ($p=0.68$). Mean maximal glucose level was 146.4 and 145.2 mg/dL for Phase I and Phase II, respectively ($p=0.85$). Mean maximal glucose level increment was similar for the entire 6-hour lasting fasting and protein load test (26.6 mg/dL vs. 27.6 mg/dL, respectively, $p=0.78$). There was no difference between the change in baseline vs. 6th hour glucose levels for the fasting state vs. protein load test (12.5 mg/dL and 19 mg/dL, respectively, $p=0.04$). The glucose variability assessed by CGMS-based standard deviation of mean glucose levels was 36.4 and 37.9 mg/dL for Phase I and Phase II, respectively ($p=0.01$).

Conclusion: The administration of a pure protein load does not seem to have a clinically significant impact on glucose levels in T1DM patients treated with insulin pumps. Thus, we do not recommend protein content counting for glycemic control in this group of patients.

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Effects of insulin degludec and insulin glargine on day-to-day fasting blood glucose variability in patients with type 1 diabetes

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Background and aims: Insulin degludec (IDeg), a novel ultra-long acting insulin analogue, has been suggested to have a longer and flatter glucose-lowering effect than insulin glargine (IGlar). It is thus possible that IDeg yields lower glycemic variability, in particular at fasting state. We have compared the effects of IDeg and IGlar on day-to-day variability of fasting blood glucose (FBG) in type 1 diabetes subjects treated with basal-bolus insulin injections.

Materials and methods: This open-label, multicenter, randomized crossover trial investigated the effects of 4-week basal-bolus insulin treatment with either IDeg or IGlar as a basal insulin. Patients who were found to satisfy the inclusion criteria were randomly allocated IGlar (first period) / IDeg (second period) or IDeg (first period) / IGlar (second period) groups. The last week of each treatment period was the data-collecting phase during which the patients were directed to examine blood glucose level 7 times per day. The level of glycoalbumin was measured at the end of each treatment period. The primary end point was the standard deviation (SD) and the coefficient of variation (CV) of FBG during the last week of the treatment period. Secondary end points included the level of glycoalbumin, the dose of daily insulin, the intra-day variability of blood glucose level, and the frequency of severe hypoglycemia. Data are shown as means \pm SD, and were statistically analyzed with the paired t-test.

Results: Thirty-six randomized subjects (17 in the IDeg/IGlar and 19 in the IGlar/IDeg groups) were recruited, and the data of 32 subjects who completed the trial were analyzed (age: 57.4 ± 13.9 years, BMI: 22.6 ± 3.2 kg/m², HbA1c: 7.4 ± 0.8 %). The mean (139.5 ± 31.8 vs. 154.2 ± 37.2 mg/dL, $P = 0.03$) and the SD (46.9 ± 17.5 vs. 57.5 ± 24.6 mg/dL, $P = 0.02$) of FBG were smaller during the IDeg treatment period than those during the IGlar treatment period whereas the CV was not different between the two periods (IDeg 34.3 ± 13.3 vs. IGlar 37.1 ± 13.0 mg/dL, $P = 0.29$). The level of glycoalbumin was similar in the two treatment periods (IDeg 21.5 ± 3.0 vs. IGlar 21.8 ± 3.6 mg/dL, $P = 0.39$). The daily dose of basal insulin in the IDeg period was slightly but significantly smaller than that in the IGlar period (IDeg 11.0 ± 5.2 vs. IGlar 11.8 ± 5.6 units/day, $P < 0.01$). The SD of the 7 measurements of blood glucose was similar in the two treatment periods (IDeg 56.4 ± 15.3 vs. IGlar 61.0 ± 16.9 mg/dL, $P = 0.19$). No severe hypoglycemia was observed during the trial period.

Conclusion: In patients with type 1 diabetes, IDeg yielded lower FBG levels with lower day-to-day variability of FBG as compared to IGlar. IDeg is thus a useful option for the supplementation of basal insulin in type 1 diabetes.

Clinical Trial Registration Number: UMIN000009965

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Low within- and between-day variability in exposure to new insulin glargine 300 U/ml

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Background and aims: Low fluctuation and high reproducibility in exposure are needed for effective and safe basal insulin use.

Materials and methods: Fifty people with type 1 diabetes mellitus underwent two 24-h euglycaemic clamps in steady state conditions after 6 QD administrations of 0.4 U.kg⁻¹ in a double-blind, randomised, two-way crossover study demonstrating equivalence in bioavailability of new insulin glargine 300

U.ml⁻¹ (Gla-300) formulations (R, standard cartridge; T, vial formulation). Serum insulin (INS) concentration was analysed by radioimmunoassay.

Results: The INS-AUC point estimate of T/R was 1.00 (90% CI: 0.95 to 1.06). Due to low fluctuation in INS-concentration over time (Table), median cumulative exposure (INS-AUC₀₋₂₄/INS-AUC₂₄) developed linearly over 24 h, and excursion (BDE = $[C_{\max} - C_{\min}]/2$) from the average concentration within 24 h ($C_{\text{AVG}} = \text{INS-AUC}_{24}/24$) was only 3.3 $\mu\text{U.ml}^{-1}$. Within-day variability (fluctuation; peak-to-trough ratio [PTR] = C_{\max}/C_{\min}) was <2 . SWING ($[C_{\max} - C_{\min}]/C_{\text{AVG}}$) = PTR-1) and peak-to-trough fluctuation (PTF = $[C_{\max} - C_{\min}]/C_{\text{AVG}}$) were <1 . Between-day variability (reproducibility; within-subject coefficient of variation for exposure, CV% [INS-AUC]), was 17.4% (90% CI: 14.9 to 21.1), at a between-subject CV% (INS-AUC) of 34.8% (90% CI: 28.8 to 43.4).

Conclusion: Gla-300 appears suitable for safe, effective basal insulin use, providing evenly distributed 24-h coverage at a clinically relevant dose of 0.4 U.kg⁻¹ due to low fluctuation and high reproducibility in insulin glargine exposure.

Table

	C _{max} [$\mu\text{U.ml}^{-1}$]	C _{AVG} [$\mu\text{U.ml}^{-1}$]	C _{min} [$\mu\text{U.ml}^{-1}$]	BDE [$\mu\text{U.ml}^{-1}$]	PTR	SWING	PTF
N	99	99	99	99	90	90	99
Median (Interquartile range)	15.0 (12.9–18.2)	11.3 (9.5–13.9)	8.2 (6.5–10.3)	3.3 (2.7–4.7)	1.8 (1.6–2.1)	0.8 (0.6–1.1)	0.6 (0.4–0.8)

Clinical Trial Registration Number: NCT01838083

Supported by: Sanofi.

954

Simulating the glycaemic impact of meal insulin dose determinations using continuous glucose monitoring vs self-monitored blood glucose

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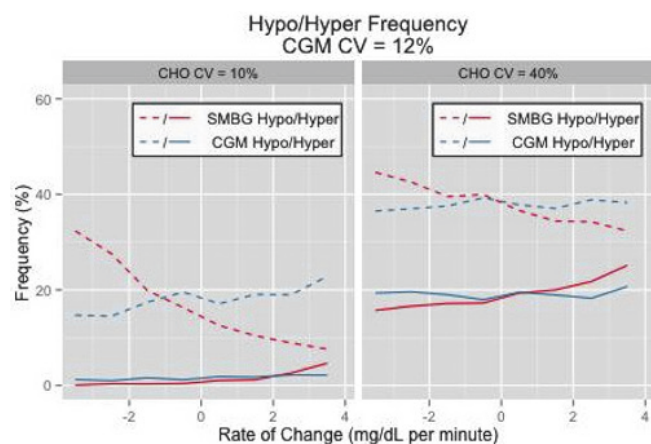
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Background and aims: Current continuous glucose monitoring (CGM) systems are labeled as adjunctive to self-monitored blood glucose (SMBG). The user guides provide instructions that diabetes management decisions should be based on blood glucose values. However, there are many anecdotes and emerging data that some CGM users make their dosing decisions based on the CGM data. This Monte Carlo simulation compares the impact of determining a pre-meal insulin dose using CGM and SMBG glucose on the frequency of post-meal hypoglycemia (< 70 mg/dL, 3.8 mmol/L) and hyperglycemia (> 180 mg/dL, 10.0 mmol/L).

Materials and methods: The dose is calculated from pre-meal glucose and estimated carbohydrate (CHO) count and targets a post-meal glucose of 100 mg/dL (5.6 mmol/L), allowing CGM users an adjustment based on the direction and rate of glucose change (ROC). The study quantifies the impact of this adjustment at different magnitudes of CHO counting errors and assumes correct dosing parameters. The evaluation is based on simulated patient data across ranges of pre-meal glucose (50 to 400 mg/dL, 2.8 to 28 mmol/L), ROC (-4 to 4 mg/dL/minute, -0.2 to 0.2 mmol/L/minute) and CHO (30 to 100 grams). The dose and post-meal glucose are calculated using a simplified but realistic model and allows comparison of hypoglycemia and hyperglycemia frequencies for the two monitoring methods. The model considers the differences in device accuracy and precision. However, it does not consider the impact of outlier glucose values (from either SMBG or CGM) or include the use of CGM alerts.

Results: Both methods perform similarly when ROC is close to 0. With CGM, the frequency of hypoglycemia and hyperglycemia changes little across the ROC range. At negative values of ROC, SMBG results in a higher frequency of hypoglycemia and a lower frequency of hyperglycemia, compared to CGM. The opposite is true at positive ROC. As CHO error increases, so does the frequency of hyperglycemia and hypoglycemia, and the differences between dosing decisions using CGM and SMBG are minimized.

Conclusion: Similar to carbohydrate counting errors, the direction and ROC at the time of the insulin dose can have major impacts on subsequent rates of hypoglycemia and hyperglycemia. Accordingly, in order to optimize glycemic control, the direction and ROC should be considered when determining a meal insulin dose. This model suggests that using CGM data to determine a dose has glycemic advantages relative to using a static SMBG measurement.



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Least glucose variability is observed with the combination of a GLP-1 agonist and basal insulin among four commonly used insulin regimens in type 2 diabetes (VARIATION study)

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Background and aims: The majority of insulin management trials to date have focused on HbA1c as the primary measure of glycemic control. Limited data pertaining to glucose variability with various insulin regimen exists in the literature. This cohort study compares glucose variability in patients with well-controlled type 2 diabetes (DM) among four commonly-used insulin regimen (A= basal insulin + oral drugs, B= basal insulin + GLP-1 agonist, C= pre-mix insulin and D= basal-bolus insulin).

Materials and methods: Eighty patients were recruited from five of our diabetes clinics in Ontario, Canada between November 2013 and March 2014. Study inclusion criteria were 1. type 2 DM; 2. age between 18 to 80 years; 3. BMI ≤ 45 kg/m²; 4. stable insulin regimen for a minimum of 6 months; 5. clinically stable HbA1c value of $\leq 7.5\%$ within 3 months before study enrolment. Participants were instructed to follow usual diet and exercise regimen during the 6-day, masked Continuous Glucose Monitoring (CGM) period. Daily glucose standard deviation (SD) was chosen as the primary outcome for the study. Six patients were excluded from analysis (three for clinical instability; three for missed medication doses during 6-day CGM period).

Results: Key baseline clinical characteristics as well as CGM results are presented in Table 1. Each of the four study cohorts had an average HbA1c of 7.0%. Statistically significant differences were observed on inter-cohort comparisons on Analysis of Covariance (ANCOVA) modeling - with the lowest SD observed in cohort B compared to cohorts C and D (1.8 mmol/L vs. 2.1 and 2.2 mmol/L; $p=0.05$ and 0.01 , respectively). SD of cohort B was also numerically less compared to that for cohort A (2.0 mmol/L), however this did not reach statistical significance ($p=0.15$). Self-reported hypoglycemia episodes during the 6-day study period were highest among cohort D compared to the other three cohorts ($p=0.01$ vs. cohorts A & B; $p=0.03$ vs. cohort C). No other statistically significant differences were observed on inter-cohort comparisons for CGM measurements (frequency, duration and degree of hypoglycemia or hyperglycemia).

Conclusion: Among patients with Type 2 DM who have well-controlled HbA1c on insulin, least glucose variability is observed in the combination regimen using basal insulin with a GLP-1 agonist. This reduced glucose variability with GLP-1 agonists suggests their complementary glycemic action with basal insulin and is explained by known mechanisms of action for this incretin-based injectable medication class - with multi-pronged effects on both alpha and beta cells in the pancreas as well as appetite suppression.

	Cohort A = Basal insulin + oral	Cohort B = Basal insulin + GLP-1	Cohort C = Pre-mixed insulin	Cohort D = Basal-bolus insulin
Age in years	64 (11)	60 (9)	65 (10)	61 (10)
HbA1c prior to CGMS (%)	7.0 (0.4)	7.0 (0.2)	7.0 (0.4)	7.0 (0.4)
BMI in kg/m ²	27.8 (6)	31.1 (5)	30.6 (5)	29.8 (4)
Duration of diabetes in years	16 (10)	14 (7)	19 (7)	18 (8)
Self-reported hypoglycemia during 6-day study period	5 episodes	3 episodes	8 episodes	34 episodes**
	None ≤ 3 mmol/L	None ≤ 3 mmol/L	1 episode ≤ 3 mmol/L	8 episodes ≤ 3 mmol/L

CGM data - Daily measure ²				
Daily average SD (mmol/L)	2.0 (0.6)	1.8 (0.5)*	2.1 (0.5)	2.2 (0.6)
CGM data - Daily measure ² - Hypoglycemia ³				
Frequency†	0.3 (0.5)	0.2 (0.7)	0.3 (0.5)	0.3 (0.3)
Percentage of time†	2.2 (3.5)	2.1 (5.0)	1.8 (3.7)	2.8 (3.7)
CGM data - Daily measure ² - Hyperglycemia ⁴				
Frequency	2.4 (0.5)	2.4 (0.8)	2.7 (0.7)	2.3 (0.7)
Percentage of time	40.4 (12.7)	38.1 (21.5)	51.0 (15.3)	40.6 (17.4)
AUC above threshold†	0.8 (0.7)	0.6 (0.6)	0.9 (0.9)	0.8 (0.9)

†Results are presented as Mean (SD), unless denoted † when they represent median (IQR)

²Daily measures were the averages calculated from a continuous 6-day monitoring period.

³Hypoglycemia was defined as the incidence when blood glucose level was equal to or below the threshold of 3.9 mmol/L

⁴Hyperglycemia was defined as the incidence when blood glucose level was above the threshold of 7.8 mmol/L.

* denotes statistical significance compared to cohorts C and D

** denotes statistical significance compared to cohorts A, B and C

SD = standard deviation, IQR = inter-quarter range, AUC = area under the curve.

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Sodium glucose co-transporter-2 (SGLT2) inhibitor empagliflozin (EMPA) in type 1 diabetes (T1D): impact on diurnal glycaemic patterns

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Background and aims: We recently reported improved glycemic control, decreased hypoglycemia incidence, and reduced insulin dose in T1D patients treated with open-label EMPA. To further characterize the effects, we analysed glucose patterns by continuous glucose monitoring (CGM).

Materials and methods: In this 8-week single-arm open-label pilot study of EMPA (NCT01392560), we compared ambulatory glucose profiles (AGP) during 2-week intervals in a placebo run-in, end-of-treatment, and post-treatment period. Glucose exposure was measured by total area under the curve (AUC_{TOTAL} in mg/dL-h), AUC_{NIGHT} (23:00 to 6:59h) and AUC_{DAY} (7:00 to 22:59h). Glucose Variability was represented by inter-quartile range and Glucose Stability by the average hourly absolute change (in mg/dL/hr) of all CGM readings in the 2-week periods.

Results: 40 patients aged 24 ± 5 years, and A1c $8.0 \pm 0.9\%$ completed the trial. 39 patients had data available for the analysis of the CGM endpoints. Though A1c decreased ($8.0 \pm 0.9\%$ to $7.6 \pm 0.9\%$, $p < 0.0001$), AUC_{TOTAL} decreased non-significantly from 153.7 ± 25.4 to 149.0 ± 30.2 mg/dL-h ($p=0.31$) and increased post-treatment (164.1 ± 29.5 mg/dL-h, $p=0.02$ vs. baseline). The trend of decrease in AUC_{NIGHT} (152.0 ± 36.6 to 141.9 ± 34.4 mg/dL-h, $p=0.13$) exceeded AUC_{DAY} (154.5 ± 24.5 to 152.6 ± 30.4 mg/dL-h, $p=0.65$). Glucose Variability showed a decreasing trend (from 83.1 ± 18.9 to 75.6 ± 28.6 mg/dL, $p=0.06$) more than Glucose Stability (from 10.8 ± 3.6 to 10.3 ± 4.5 mg/dL/h, $p=0.51$), and subsequently levels increased relative to pre-treatment levels (89.3 ± 19.3 , $p=0.04$ and 11.8 ± 3.7 , $p=0.08$) when EMPA was discontinued. The on-treatment changes occurred despite basal insulin reduction (25.7 ± 10.6 to 19.5 ± 7.9 U, $p < 0.0001$) without significant bolus reduction (29.0 ± 15.8 to 27.0 ± 14.2 U, $p=0.19$).

Conclusion: The improved A1c observed with EMPA was associated with a pattern of improved night-time glycaemia more prominent than daytime.

Clinical Trial Registration Number: NCT01392560

Supported by: Boehringer Ingelheim

PS 077 Old and new insulin formulations

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A single dose pharmacokinetic study of basal insulin peglispro in subjects with hepatic impairment

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Background and aims: Basal insulin peglispro (BIL; LY2605541) is being developed for the treatment of type 1 and type 2 diabetes mellitus (T2DM) as a once daily subcutaneous (SC) administration. BIL is a novel, PEGylated insulin lispro that has a large hydrodynamic size. It has prolonged duration of action which is related to a delay in insulin absorption and a reduction in clearance. The patient population may include those with hepatic impairment, so the pharmacokinetics (PK) of BIL was assessed in subjects with varying degrees of hepatic impairment compared to healthy subjects with normal hepatic function.

Materials and methods: In this parallel-group, open-label study, 35 subjects received a single SC dose of 0.33 U/kg (0.075 mg/kg) BIL and blood samples for PK were taken up to 9 days postdose. BIL PK was determined using a specific, validated enzyme-linked immunosorbent assay method. PK parameters were calculated using noncompartmental analyses. The effect in subjects with hepatic impairment was compared to subjects with normal hepatic function using the ratio of least squares (LS) geometric means of primary PK parameters: area under the concentration time curve from zero to infinity ($AUC(0-\infty)$) and maximum observed drug concentration (C_{max}). $AUC(0-\infty)$ and C_{max} were log transformed and analyzed using an Analysis of Variance model with group as a factor.

Results: In total, 35 subjects were assessed (12 with normal hepatic function; 8 mild, 8 moderate, 7 severe hepatic impairment, based on the Child-Pugh criteria). Subjects were nondiabetic, apart from one subject with T2DM (moderate hepatic impairment). Subjects were male (57%) and female (43%), 41–69 years old, with mean body mass index (SD) 27.7 kg/m² (4.52 kg/m²). Healthy subjects were matched to hepatically impaired subjects for age (± 10 years), weight (± 10 kg), and gender. Ratios of the LS geometric means and 90% confidence intervals (CIs) for $AUC(0-\infty)$ for mild, moderate, and severe hepatically impaired subjects to the healthy subjects were 0.789 (0.556, 1.12), 0.744 (0.525, 1.06), and 0.782 (0.543, 1.12), respectively. Ratios (90% CIs) for C_{max} were 0.728 (0.398, 1.33), 0.905 (0.495, 1.65), and 0.840 (0.448, 1.58), respectively. Median time of C_{max} and mean half-life were similar between the hepatically impaired subjects and healthy subjects. Healthy subjects reported 7 treatment emergent adverse events (TEAEs) (5 of 12 subjects; 41.7%) and hepatically impaired subjects reported 7 TEAEs (1 TEAE reported by 1 of 8 [12.5%] mild; 3 TEAEs reported by 2 of 8 [25.0%] moderate, and 3 TEAEs reported by 2 of 7 [28.6%] severe). The majority of TEAEs were mild in severity. TEAEs experienced by >1 subject included hypoglycemia, nausea, fatigue, and headache.

Conclusion: Single doses of BIL were well tolerated by hepatically impaired and healthy subjects in this study. A substantial overlap of $AUC(0-\infty)$ and C_{max} was observed across all groups. Although the mean $AUC(0-\infty)$ and C_{max} were lower in hepatically impaired subjects, the ratios between hepatically impaired and healthy subjects were not statistically different. Based on data from this study, requirement for dose adjustment of BIL in subjects with hepatic impairment would not be anticipated.

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Supported by: Eli Lilly and Company

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Conventional insulin therapy (CT) vs multiple insulin injection (MIT) in type 2 diabetes: analysis of a quality assurance project (DPV)

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Background and aims: People with type 2 diabetes and insulin therapy receive predominantly an intensive form of insulin treatment with multiple insulin injections (MIT). Evidence from randomized studies for the superiority of MIT over CT in type 2 diabetes is lacking, but MIT produced increased weight gain, higher costs of therapy and more hypoglycaemic episodes. This survey provides data from a project of quality assurance in Germany and Austria (DPV) with documentation of insulin treatment in type 2 diabetes.

Materials and methods: Patients older than 18 years with type 2 diabetes and insulin treatment were extracted from the DPV database. CT was defined as 2 insulin injection time-points per day, MIT as more than 2 insulin injection time-points per day. Patients with less than 2 daily insulin injection time-points and patients with LADA (positive β -cell antibodies) were excluded from the analysis. Patients with insulin therapy and Metformin as concomitant therapy were included, but not evaluated separately.

Results: A total of 88 519 (CT 23 118 (26%); MIT 65 401 (74%)) patients were analysed. Patients with CT (44% male) compared to patients with MIT (53% male) were older (74.6 (10.2) vs. 66.7 (12.0) years), had comparable time from diabetes diagnosis (13.0 (9.5) vs. 13.4 (9.8) years), lower BMI (29.1 (5.7) vs. 31.7 (6.7) kg/m²), lower daily insulin dose (39.2 (20.2) vs. 65.3 (41.1) IE/day) and better HbA_{1c} (7.81 (1.9) vs. 8.14 (2.0)%; $p < 0.001$; normal mean 5.05%). Hypoglycaemic events (per patient/year) with need for help was comparable in both groups (1.02 (1.07) vs. 1.06 (1.24)), hypoglycaemia with coma was associated more often with CT (0.051 (0.43) vs. 0.038 (0.38)).

Conclusion: In a non randomized, multicentric, observational survey with data derived from clinical routine care, glycaemic control of people with CT was comparable to people with MIT. Patients with MIT were predominantly male, younger, had higher BMI and needed more insulin. Hypoglycaemic episodes had a similar incidence compared to other studies in patients with insulin-treated type 2 diabetes.

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Preserved pharmacokinetic properties and distinct glycaemic effects of insulin degludec and liraglutide in IDegLira, a fixed ratio combination product

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Background and aims: Insulin degludec/liraglutide (IDegLira) is a novel fixed ratio combination of insulin degludec (IDeg), a basal insulin with an ultra-long duration of action, and liraglutide (Lira), a once-daily glucagon-like peptide-1 analogue. The pharmacokinetics (PK) and pharmacodynamics of IDegLira were compared with those of the mono-components in healthy volunteers and in subjects with type 2 diabetes.

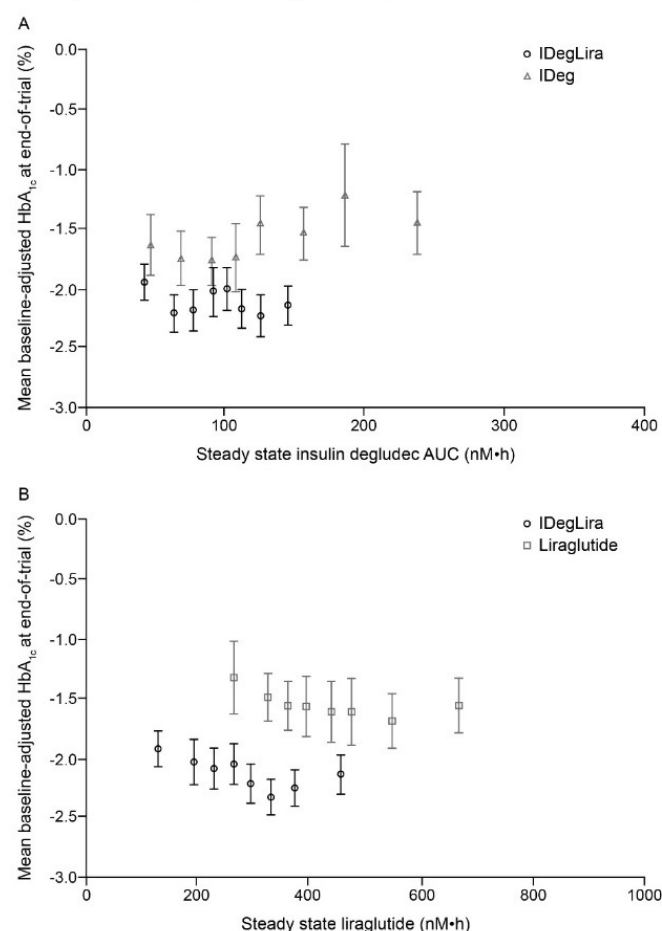
Materials and methods: The relative bioavailability of IDegLira and its mono-components were compared in a single-dose, randomised, double-blind, double-dummy four-period crossover clinical pharmacology study in 24 healthy volunteers. Dose proportionality and covariate effects on exposure were evaluated in a population PK analysis of data from a randomised treatment-to-target phase 3 study (n=1549) in subjects with type 2 diabetes. The exposure-response relationship for change from baseline in HbA_{1c} of IDegLira relative to mono-components was also assessed using phase 3 data.

Results: The overall PK properties of IDeg and Lira were preserved for IDegLira compared with IDeg or Lira when dosed separately. The IDeg exposure (area under the curve) was equivalent when dosed as IDegLira or IDeg alone. Lira AUC was lower when dosed as IDegLira but met the criterion for bioequivalence (90% CI within 0.8–1.25). Population PK analysis revealed no relevant deviations from dose proportionality for the components of IDegLira, and covariate effects on exposure (such as body weight) were consistent with previous results for IDeg and Lira. Glycaemic response to IDegLira was larger than that of IDeg or Lira alone, reflecting distinct glucose-lowering

effects of its mono-components throughout the dose/exposure range (see Figure).

Conclusion: The PK properties of IDeg and Lira are preserved in the IDegLira co-formulation; both mono-components contribute to glycaemic control across the recommended dose range.

Change in HbA_{1c} from baseline to Week 26 (A) IDegLira and IDeg versus exposure of IDeg and (B) IDegLira and Lira versus exposure of Lira



IDegLira, IDeg and Lira are represented by circles, triangles and squares, respectively. HbA_{1c} is expressed as baseline-adjusted mean change with 95% CI. Exposure is depicted as percentiles of AUC at steady state.

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Aspart pharmacokinetics in children with type 1 diabetes during closed loop insulin delivery: comparison between diluted (U20) and standard (U100) insulin strengths

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Background and aims: To compare pharmacokinetics of two different concentrations of insulin aspart in children aged 3 to 6 years with type 1 diabetes (T1D) during closed loop insulin delivery.

Materials and methods: Young children with T1D underwent an open-label, randomized, two-period crossover study involving two occasions in a clinical research facility, 2 to 6 weeks apart, from 17:00 day 1 to 8:00 day 2 with an evening meal consumed at 17:00. In random order, diluted (1:5 dilution with normal saline, 20 IU/ml) or standard strength (100 IU/ml) insulin aspart was administered via an insulin pump as a meal bolus and then overnight during closed loop advised by a model predictive algorithm. Plasma insulin was measured every 30 to 60 min. A compartment model adopting Bayesian parameter estimation was used to measure time-to-peak of insulin concentra-

tion (t_{max}), insulin metabolic clearance rate (MCR_I), and background insulin concentration (ins_c).

Results: Eleven children [6 male; age 5.07(range 3.75-6.96yrs); A1C 60(14) mmol/mol; BMI sds 1.0(0.8); duration of diabetes 2.2(1.0)yrs; mean (SD); total daily dose 12.9(10.6,16.5)IU; fasting C-peptide 5(5,17.1)pmol/l; median(IQR)] participated in the study. Pharmacokinetic parameters (Table) were not different between the two aspart concentrations but t_{max} showed less inter-subject variability following administration of diluted aspart (SD 8.7 vs. 14.4min; diluted vs. standard strength; $P=0.047$). ins_c but not the other parameters was positively correlated with total daily insulin dose (Spearman's $r_s = 0.7$; $P=0.014$) but not with fasting C-peptide ($r_s = -0.19$; $P=0.39$) suggesting that it does not reflect residual insulin secretion.

Conclusion: Diluting insulin aspart does not change its pharmacokinetics, but may result in less variable absorption and could be used in young people with T1D undergoing closed loop insulin therapy.

Table. Comparison of parameter estimates following administration of standard strength (100IU/ml) and diluted (20IU/ml) insulin aspart

Model parameter	Standard (n=11)	Diluted (n=11)	P value
t_{max} (min)	59.2 (14.4)	61.6 (8.7)	0.59 ^a
MCR_I ($10^{-2} \times l/kg/min$)	1.98 (0.99)	1.89 (0.82)	0.47 ^a
ins_c (mU/l)	5.6 (0.2, 12.0)	3.9 (0.5, 10.9)	0.66 ^b

Parameter estimates are presented as mean (SD) or median (IQR) as appropriate

^a paired t-test.

^b Wilcoxon signed rank test.

Clinical Trial Registration Number: NCT01557634

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New insulin glargine 300 U/ml: efficacy and safety of flexible vs fixed dosing intervals in people with type 2 diabetes mellitus

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Background and aims: The smoother, more prolonged PK and PD profiles of new insulin glargine (300 U/ml; Gla-300) vs glargine 100 U/ml (Gla-100), with glycaemic control extending beyond 24 h, provide a rationale for flexible timing of daily injections depending on individual lifestyle. These 3-month sub-studies, of Gla-300 injected each evening, compared the efficacy and safety of using a fixed 24-h dosing interval with a flexible dosing regimen allowing between-injection intervals of 24 ± 3 h on at least 2 days each week.

Materials and methods: The background multicentre, 6-month, open-label studies compared Gla-300 vs Gla-100 in people with type 2 diabetes mellitus using basal + meal-time insulin (EDITION 1) or basal insulin + OADs (EDITION 2). Participants (EDITION 1, N=109; EDITION 2, N=89) using Gla-300 were randomised at month 6 to continue the fixed regimen or move to flexible intervals. Efficacy and safety were evaluated 3 months later (month 9).

Results: The frequency of maintaining a 24 ± 1 h interval between injections was ~88% with the fixed regimen and ~60% with the flexible regimen. HbA_{1c} change (primary endpoint) was comparable in fixed vs flexible regimens (Table). Percentages of participants with ≥ 1 hypoglycaemia at any time of day (24 h), or ≥ 1 nocturnal (00:00-05:59 h) hypoglycaemia, were also comparable. Severe hypoglycaemia was experienced by only 1 participant, in the EDITION 1 sub-study. There were no differences in adverse events.

Conclusion: Occasional flexibility in timing of daily Gla-300 injections by \pm 3 h resulted in similar efficacy and safety compared with advising injections at a fixed time each day.

Table: Outcomes of flexible and fixed dose intervals in the two sub-studies

		EDITION 1		EDITION 2	
		Flexible	Fixed	Flexible	Fixed
Randomised population, <i>n</i>		56	53	45	44
mITT population, <i>n</i>		55	53	44	42
HbA _{1c} , %	Baseline*	7.21 (0.91)	7.17 (0.89)	7.41 (0.96)	7.47 (1.05)
	Change† [months 6–9]	0.21 (0.11)	0.15 (0.12)	−0.12 (0.15)	−0.25 (0.16)
	Difference‡	0.05 (−0.19 to 0.30)		0.13 (−0.15 to 0.42)	
		56	53	44	43
Safety population, <i>n</i>		56	53	44	43
Hypoglycaemic events, <i>n</i> (%)	All, any time of day [24 h]	32 (57.1)	35 (66.0)	16 (36.4)	18 (41.9)
	All, nocturnal [00:00–05:59 h]	15 (26.8)	12 (22.6)	7 (15.9)	10 (23.3)
	Severe, any time of day [24 h]	0 (0)	1 (1.9)	0 (0)	0 (0)
		56	53	44	43
Percentage of injections within dosing intervals, % (SD)	24 \pm <1 h	63.4 (26.6)	87.7 (22.2)	53.1 (27.2)	88.8 (20.5)
	24 \pm 1 to 2.5 h	13.5 (15.2)	8.4 (16.2)	18.9 (20.9)	8.8 (17.1)
	24 \pm >2.5 h	23.0 (26.6)	3.9 (11.1)	28.0 (24.4)	2.4 (8.9)

*Means (SD); †LS means (SE); ‡LS mean difference (95% CI).
mITT, modified intention-to-treat.

Clinical Trial Registration Number: NCT01499082, NCT01499095

Supported by: Sanofi.

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Unmet needs in HbA_{1c} goal achievement (< 7.0%) by patients with type 2 diabetes mellitus treated with basal insulin: pooled randomised controlled trial data and real-world clinical practice data

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Background and aims: Despite the efficacy of basal insulin therapy in patients with type 2 diabetes mellitus (T2DM), many patients do not reach glycaemic goals. Here we use both randomized clinical trial (RCT) data and “real-world” data from a retrospective study of electronic medical records (EMR) to assess the characteristics of T2DM patients who do not achieve target glycaemic control while on basal insulin (HbA_{1c} \geq 7.0%) in order to better understand the clinical characteristics of these patients.

Materials and methods: Patients with T2DM aged \geq 18 years treated with basal insulin, but with HbA_{1c} \geq 7.0% were identified by analyzing results from 11 RCTs at 6 months after the index date, defined as date of first prescription for basal insulin, and results from the GE Centricity EMR database at 6-months and at 12-months follow-up after the index date. Patients were stratified based on fasting plasma glucose (FPG) levels (< 130 or \geq 130 mg/dL). Information on patient demographic and clinical characteristics were available from both sources of data.

Results: The table shows that in the RCTs around 52% achieved HbA_{1c} < 7.0% (recommended by the ADA for most diabetic patients). HbA_{1c} < 7.0% was achieved by fewer patients (~27%) in the EMR database at both 6 and 12 months. Among those with HbA_{1c} \geq 7.0%, 54.9% of RCT patients and 17.6% and 27.7% of EMR patients at 6 and 12 months, respectively, had FPG < 130 mg/dL. About half of the RCT patients not achieving goal had a FPG \geq 130 mg/dL. This suggested that these patients needed further basal insulin titration, whereas patients at goal likely required postprandial glucose control. In the EMR cohort, > 70% of patients were likely to need additional basal insulin titration.

Conclusion: Failure to adequately titrate basal insulin is an unmet need in many T2DM patients, which is evident even in RCTs. When basal insulin is adequately titrated and FPG is controlled, additional postprandial treatment may be needed. Another important unmet need is understanding the causes of failure to achieve control of FPG with basal insulin.

n (%)	All Patients		Patients with HbA _{1c} > 7% and FPG Data	
	HbA _{1c} < 7%	HbA _{1c} \geq 7%	FPG < 130 mg/dL	FPG \geq 130 mg/dL
RCT 6 months	1600 (51.9)	1482 (48.1)	797 (54.9)	656 (45.1%)
EMR 6 months	3464 (27.6)	9098 (72.4)	1938 (17.6)	9098 (82.4)
EMR 12 months	3805 (27.1)	10233 (72.9)	2382 (27.7)	6221 (72.3)

Supported by: Sanofi US, Inc.

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New insulin glargine 300 U/ml: glycaemic control and hypoglycaemia in a meta-analysis of phase 3a EDITION clinical trials in people with type 2 diabetes mellitus

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Background and aims: The EDITION 1, 2 and 3 studies compared the efficacy and safety of new insulin glargine 300 U/ml (Gla-300) with insulin glargine 100 U/ml (Gla-100) in people with type 2 diabetes mellitus on basal and mealtime insulin, basal insulin and OADs, and no prior insulin, respectively. A meta-analysis of these studies was conducted to study efficacy and safety.

Materials and methods: Meta-analysis of these three studies enabled glycaemic control and hypoglycaemia to be examined over 6 months in a large, heterogeneous type 2 diabetes mellitus population (Gla-300, N=1247; Gla-100, N=1249).

Results: Mean change in HbA_{1c} was comparable for Gla-300 and Gla-100 (LS mean change [SE]: −1.02 [0.03] % for both groups). Gla-300 was associated with reduced risk of experiencing hypoglycaemia compared with Gla-100 (nocturnal and at any time of day; Table). Nocturnal hypoglycaemic event rates were consistently lower with Gla-300 than Gla-100. Severe hypoglycaemia was rare in both treatment groups (2.3% with Gla-300 vs 2.6% with Gla-100). Weight gain with Gla-300 and Gla-100 was slight (LS mean change [SE]: 0.49 [0.10] kg and 0.75 [0.10] kg, respectively), with a trend for less weight gain with Gla-300 (LS mean difference: −0.26 [95% CI: −0.52 to 0.01] kg, *p*=0.058).

Conclusion: Gla-300 provides comparable glycaemic control to Gla-100 in type 2 diabetes mellitus, with consistently less hypoglycaemia at any time of the day and less nocturnal hypoglycaemia.

Table – Glycaemic control and hypoglycaemic events over 6 months in a meta-analysis of the EDITION 1, 2 and 3 studies

		HbA _{1c} (%)*	
<i>mITT population</i>		Gla-300 (N=1239)	Gla-100 (N=1235)
Baseline	Mean	8.30	8.31
Change from baseline to month 6	LS mean (SE)	-1.02 (0.03)	-1.02 (0.03)
		Weight (kg)**	
<i>Safety population</i>		Gla-300 (N=1242)	Gla-100 (N=1246)
Baseline	Mean	99.89	99.94
Change from baseline to month 6	LS mean (SE)	0.49 (0.10)	0.75 (0.10)
		Nocturnal hypoglycaemia (00:00–05:59 h)	
<i>Safety population</i>		Gla-300 (N=1242)	Gla-100 (N=1246)
Any hypoglycaemia		Hypoglycaemia at any time of day (24 h)	
Baseline to month 6	% people ≥1 event	31.7	41.3
	RR* (95% CI)	0.77 (0.69 to 0.85)	0.92 (0.87 to 0.96)
	Events/participant-year	2.25	3.30
	RR* (95% CI)	0.68 (0.57 to 0.82)	0.85 (0.76 to 0.96)
Confirmed (<3.0 mmol/l [\leq54 mg/dl]) or severe hypoglycaemia			
Baseline to month 6	% people ≥1 event	9.7	13.2
	RR* (95% CI)	0.73 (0.59 to 0.91)	0.81 (0.72 to 0.90)
	Events/participant-year	0.37	0.56
	RR* (95% CI)	0.67 (0.50 to 0.91)	0.93 (0.76 to 1.13)
Confirmed (\leq3.9 mmol/l [\leq70 mg/dl]) or severe hypoglycaemia			
Baseline to month 6	% people ≥1 event	30.0	39.8
	RR* (95% CI)	0.75 (0.68 to 0.83)	0.91 (0.87 to 0.96)
	Events/participant-year	2.10	3.06
	RR* (95% CI)	0.69 (0.57 to 0.84)	0.86 (0.77 to 0.97)
Severe hypoglycaemia			
Baseline to month 6	% people ≥1 event	0.6	1.0
	RR* (95% CI)	0.71 (0.32 to 1.59)	0.85 (0.52 to 1.39)
	Events/participant-year	0.02	0.03
	RR* (95% CI)	0.70 (0.35 to 1.42)	0.98 (0.51 to 1.86)

CI, confidence interval; mITT, modified intention-to-treat; *MMRM, mixed model for repeated measurements; RR*, relative risk; RR*, rate ratio. **LOCF, last observation carried forward.

Clinical Trial Registration Number: NCT01499082/ NCT01499095/ NCT01676220

Supported by: Sanofi

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Long-term efficacy and safety of insulin degludec in combination with bolus insulin aspart in children and adolescents with type 1 diabetes

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Background and aims: This 1:1 randomised controlled, open-label, treat-to-target trial investigated the efficacy and safety of insulin degludec (IDeg) and insulin detemir (IDet), both in combination with bolus insulin aspart (IAsp) in children and adolescents with type 1 diabetes for 26 weeks (n=174 IDeg; 176 IDet) followed by a 26-week extension (n=152 IDeg; 128 IDet).

Materials and methods: The trial included 85 children aged 1–5 years (IDeg: 43), 138 children 6–11 years (IDeg: 70) and 127 adolescents 12–17 years (IDeg: 61). Randomised subjects [baseline mean (SD) diabetes duration 4.0 (3.5) years, HbA_{1c} 8.1 (1.1)%, fasting plasma glucose (FPG) 8.7 (5.1) mmol/L], received IDeg once daily + IAsp or IDet once/twice daily + IAsp.

Results: Non-inferiority of IDeg vs. IDet at 26 weeks was confirmed for HbA_{1c} (primary endpoint): estimated treatment difference (ETD) 0.15% [-0.03; 0.32]_{95% CI} (non-inferiority margin: 0.4%, $p<0.05$). At 52 weeks, HbA_{1c} was 7.9% with IDeg and 7.8% with IDet (NS), change in mean FPG was -1.29

mmol/L with IDeg vs. +1.10 mmol/L with IDet (ETD -1.62 mmol/L [-2.84; -0.41], $p<0.05$) and mean basal insulin dose was 0.38 U/kg (IDeg) vs. 0.55 U/kg (IDet). Rates of confirmed hypoglycaemia (i.e., severe [altered mental status, cannot assist in own care, semiconscious/unconscious, or in coma \pm convulsions] and/or plasma glucose (PG) <3.1 mmol/L) were similar for IDeg and IDet at 52 weeks (57.7 vs. 54.1 events/exposure year, estimated rate ratio (ERR): 1.11 [0.89; 1.38], NS), as were rates of nocturnal (11 pm - 7 am) hypoglycaemia; (6.0 vs. 7.6 events/exposure year, ERR 0.99 [0.72; 1.34], NS). Incidence and rates of severe hypoglycaemia tended to be higher with IDeg than IDet: 17.8% vs. 13.7%; 0.51 vs. 0.33 events/exposure year (ERR 1.30 [0.64; 2.64], NS). Rates of severe hypoglycaemia, including only episodes of semiconscious/unconsciousness and coma \pm convulsions, were 0.09 and 0.14 for IDeg and IDet, respectively (ERR 0.62 [0.24; 1.60] NS, *post-hoc* analysis). The rate of hyperglycaemia episodes with ketosis (self-measured PG >14.0 mmol/L with blood ketones >1.5 mmol/L) was significantly lower for IDeg vs. IDet (0.7 vs. 1.1 events/exposure year, treatment ratio 0.41 [0.22; 0.78], $p<0.05$). Weight SD scores increased with IDeg and remained unchanged with IDet (change: 0.11 vs. -0.06, ETD: 0.17 [0.10; 0.25], $p<0.05$). Adverse event profiles were similar for IDeg and IDet.

Conclusion: IDeg+IAsp effectively improved long-term glycaemic control at lower dose, achieving significantly greater reduction in FPG versus IDet+IAsp with similar rates of overall and nocturnal hypoglycaemia and significantly lower rates of hyperglycaemia with ketosis. Rates of severe hypoglycaemia tended to be higher with IDeg than IDet. Less weight gain was observed with IDet. No safety issues were identified with IDeg or IDet.

Clinical Trial Registration Number: NCT01513473

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PS 078 Insulin immunogenicity

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Injection depth does not affect the pharmacokinetics or pharmacodynamics of insulin lispro in healthy obese or normal weight subjects

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Background and aims: An 8-mm needle length is commonly used worldwide, and recent recommendations suggest that using shorter, 4-, 5-, or 6-mm needles may be optimal for most patients to avoid intramuscular injection and still maintain adequate glucose control. The goal of this analysis was to compare the pharmacokinetics (PK) and pharmacodynamics (PD) of insulin lispro after administration at a 5-mm injection depth or an 8-mm injection depth in 2 separate populations: normal weight (Study 1) or obese (Study 2). **Materials and methods:** In both open-label, randomized, 2-period crossover euglycemic clamp studies, subjects received single doses of insulin lispro (0.25 U/kg) on 2 different occasions; PK and PD measures were collected under euglycemic conditions for up to approximately 6 hours post-dose.

Results: In Study 1, 16 healthy normal weight subjects (13 male; age 31±9 years; BMI=23±2 kg/m²) were dosed at a 5-mm and an 8-mm injection depth on 2 separate occasions. In Study 2, 16 healthy obese subjects (11 male; mean age 41±11 years; BMI=34±3 kg/m²) were also dosed at a 5-mm and an 8-mm injection depth. In both studies, PK parameters [AUC(0–∞), AUC(0–t_{last}), and C_{max}] and PD parameters (G_{tot}, R_{max}) were log-transformed prior to analysis using a mixed effects model. The table shows PK and PD parameters for both studies.

Conclusion: There were no statistical differences in PK or PD of insulin lispro following administration at the 5-mm injection depth or 8-mm injection depth in either study. Injection depths in the 5–8 mm range did not affect the PK or PD of insulin lispro in either normal weight or obese subjects. These observations are consistent with clinical data in pediatric, adult, and obese patients with type 1 and type 2 diabetes mellitus, where the 5-mm needle was found to be as efficacious and safe as an 8-mm needle.

	Study 1: Normal Weight Subjects			Study 2: Obese Subjects		
	5-mm injection depth	8-mm injection depth	Ratio of LS Means (90% CI) ^a	5-mm injection depth	8-mm injection depth	Ratio of LS Means (90% CI) ^a
N	16	16	16	16	16	16
AUC(0–∞) (pmol·min/ L)	134000 (26)	133000 (22)	1.00 (0.94, 1.07)	135000 (26)	134000 (29)	1.00 (0.95, 1.05)
C _{max} (pmol/L)	831 (30)	822 (36)	1.01 (0.94, 1.09)	569 (24)	560 (29)	0.99 (0.91, 1.07)
t _{max} (min)	60 (45–120) ^b	60 (45–120) ^b	0.00 (-60.0, 30.0) ^c	90 (30–120) ^b	90 (60–180) ^b	-15.0 (-30.0, 0.0) ^c
G _{tot} (g)	116 (24)	111 (23)	1.04 (0.95, 1.14)	129 (41.9)	130 (40.9)	0.99 (0.92, 1.08)
R _{max} (mg/min)	635 (24)	592 (19)	1.07 (0.98, 1.17)	572 (39.8)	589 (37.2)	0.97 (0.88, 1.07)
tR _{max} (min)	92.5 (45.0–220) ^b	150 (50.0–240) ^b	-32.5 (-180, 140) ^c	204 (48.0–360) ^b	237 (144–360) ^b	-21.0 (-96.0, 24.0) ^c

Abbreviations: AUC(0–∞)=area under the concentration versus time curve from zero to infinity; CI=confidence interval; C_{max}=maximum observed drug concentration; G_{tot}=total amount of glucose infused; LS=least squares; N=number of observations; R_{max}=maximum glucose infusion rate; t_{max}=time of maximum observed drug concentration; tR_{max}=time of R_{max}.

^aData are shown as geometric means and coefficients of variation unless otherwise indicated
^bmedian (range)

^cmedian of pairwise differences (range)

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Development of a clinical predictive score for requirement of hypoglycaemic agents for optimal control of blood glucose during glucocorticoid treatment

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Background and aims: Long-term glucocorticoid (GC) treatment may be necessary for patients with various autoimmune diseases. The highest dose of GC is usually administered at the initiation of the therapy, which often causes hyperglycemia and needs hypoglycemic agents in some patients. The aim of the present study is to elucidate risk factors related to GC-induced hyperglycemia and to develop a simple method to predict in advance the requirement of hypoglycemic agents to achieve optimum control of blood glucose during GC treatment.

Materials and methods: We retrospectively evaluated 230 patients (57±19 years, 57% of whom were women) with autoimmune diseases admitted between 2004 and 2013 in our department to develop a predictive scoring system. Inclusion criteria indicated that the dosage of prednisolone treatment should be more than 5 mg/day for 4 weeks at least. Exclusion criteria included use of hypoglycemic agents before GC administration or concomitant use of anti-cancer drugs.

Results: Forty-three percent (Ninety-nine persons) of GC-administered patients required hypoglycemic agents (19% for oral hypoglycemic agents, 5% for a GLP-1 analog or 20% for insulin). A logistic regression analysis revealed that risk factors of requirement of hypoglycemic agents during GC administration should be raised in accordance with age and fasting plasma glucose (FPG). A ROC analysis showed that cutoff values of age and FPG were 58 years old and 101 mg/dl, respectively. We scored patient profiles as follows: 0 point for FPG less than 101 mg/dl and age less than 58 years old, 1 point for FPG more than 101 mg/dl and age more than 58 years old, respectively. Mean of the sum of the points was 1.27 for patients requiring hypoglycemic agents and 0.64 for others, respectively. The percentages of patients requiring hypoglycemic agents in the sum of each point were 17% (12 / 72) at 0 point, 45% (48 / 106) at 1 point and 75% (39 / 52) at 2 points, respectively. The sensitivity and specificity were 87.9% and 45.8%, respectively, when 1 point was defined as the cut-off value of the sum of the scores.

Conclusion: Use of a scoring system composed in accordance with age and FPG is a simple and effective way to predict the requirement of hypoglycemic agents for hyperglycemia in patients treated with GC.

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Insulin antibodies are associated with lipotrophy in adults with type 2 diabetes mellitus

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Background and aims: The clinical significance of insulin antibodies (IA) cannot be ignored, even after the introduction of either recombinant human or analog insulin. Previous studies suggest potential associations of IA with hypoglycemia and hyperglycemia, as well as skin reactions. Furthermore, in recent studies, elevated IA levels were independent risk factors for coronary heart disease in insulin-treated elder adults, or for retinopathy in type 2 diabetic subjects with insulin therapy. In the subjects under insulin therapy, lipodystrophy, i.e. lipohypertrophy and lipotrophy, are common problems at frequently injected sites. We occasionally notice that some diabetic subjects developing lipodystrophy tend to show high levels of IA. Indeed, strong association of lipodystrophy with IA has been reported in children and adolescents with type 1 Diabetes. Herein, we endeavored to ascertain clinical and immunological factors that are associated with lipodystrophy in adults with type 2 diabetes.

Materials and methods: Subjects were inpatients of our University Hospital. The study groups consisted of 40 females and 42 males, of which mean age was 63±12 years, duration of diabetes 15±8.0 years, and HbA1c 9.0±2.2%. After obtaining approval from the ethics committee of the University and

informed consent from all subjects, blood samples were collected. IA were measured by RIA kits of IA with reduced non-specific bindings (Yamasa Co, Tokyo, Japan.) Grading of lipodystrophy was applied as follows. Grade 0: no lipodystrophy or lipohypertrophy at any injection site, grade 1: moderate lipohypertrophy without increased palpable density of subcutaneous tissue, grade 2: severe hypertrophy with increased density of the injection site, and grade 3: lipodystrophy at any injection site. Statistical significance was analysed by the Mann-Whitney U test, χ^2 test, or multiple linear regression analyses.

Results: Thirty-eight out of 82 subjects (46.3%) receiving current or previous therapy with recombinant human or analogue insulin were IA positive (percent binding $9.6 \pm 17\%$). We found lipodystrophy (grade 3) of at least one injection site in 3 subjects, severe lipohypertrophy (grade 2) in 8 and moderate lipohypertrophy (grade 1) in 23 adults. No alteration (grade 0) was found in the remaining 48 subjects. Subjects with lipodystrophy displayed significantly higher levels of percent binding with IA ($66 \pm 21\%$) than those with severe lipohypertrophy ($8.0 \pm 8.0\%$, $P=0.03$) or those without lipodystrophy ($3.3 \pm 6.3\%$, $P=0.03$). Multiple regression analyses revealed that lipodystrophy ($\beta=0.78$, $t=7.99$, $P=0.000$) and percent binding with IA ($\beta=0.65$, $t=6.05$, $P=0.000$, $R=0.65$) could significantly predict each other.

Conclusion: To our knowledge, this is the first report that showed strong relationship between lipodystrophy and IA in subjects with type 2 diabetes under insulin therapy. However, it is the chicken or the egg with lipodystrophy and percent binding with IA. Further investigations should be needed to elucidate the pathological relationship between them. In conclusion, we propose that the emergence of IA and its concomitant adverse effects should be taken into account, when starting insulin therapy in subjects with type 2 diabetes mellitus.

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The immunogenicity of originator insulin glargine: incidence and absence of clinical sequelae

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Background and aims: The immunogenicity of various basal insulins appears to be variable, with no apparent clinical impact. This analysis assessed immunogenicity and its clinical relevance in trials of the originator insulin glargine (GLA) after > 10 years experience.

Materials and methods: This was a retrospective analysis of supporting data from published registration studies of GLA vs NPH insulin (NPH) in patients with type 1 diabetes mellitus (T1DM; $n=4$) or type 2 diabetes mellitus (T2DM; $n=3$). Assessments included: change in antibody (Ab) levels (%bound/total [%B/T]) against GLA (IGAb) and human insulin (HIAb); proportion of patients with predefined change in Ab levels (≥ 20 units from baseline); and possible Ab-associated clinical effects.

Results: In the T1DM studies, baseline IGAb and HIAb levels ranged from 21.72–29.35 and 20.50–29.91 (%B/T), respectively. Significantly larger reductions in Ab levels from baseline were seen with GLA vs NPH in 2 studies for IGAb and HIAb (Table). The proportion of patients with predefined changes in Ab levels was low in all studies and treatment groups with available data: GLA, IGAb increase 2.0–14.5%, decrease 2.0–4.3% and HIAb increase 1.2–8.3%, decrease 2.8–4.3%; NPH, IGAb increase 0–1.2%, decrease 0–2.9% and HIAb increase 0–0.8%, decrease 0–2.7%. In most studies more GLA-treated patients showed an increase in IGAb; however, one study showed a decrease ($p=0.0029$). In the T2DM studies, baseline HIAb and IGAb levels ranged from 9.86–17.97 and 6.83–17.57 (%B/T), respectively. Ab level increases were less marked in GLA-treated patients (significant in 2 studies) (Table). More T2DM patients had predefined changes in Ab levels vs T1DM patients: GLA, IGAb increase 0.01–3.1%, decrease 0–6.2% and HIAb increase 0.01–2.5%, decrease 0–9.2%; NPH, IGAb increase 0.01–13.3%, decrease 0–2.8% and HIAb increase 0.01–13.8%, decrease 0–2.8%. Significantly more NPH-treated patients had predefined increases in IGAb and HIAb in Study 3002 (overall and insulin-naïve subgroup; all $p < 0.0001$). There was no evidence of insulin resistance, loss of glycaemic control, or increased hypoglycaemia associated with Ab level change in T1DM or T2DM patients. There was no association between changes in Ab levels and in HbA_{1c} in any treatment group; total insulin dose was unchanged or only slightly increased in a majority of T1DM patients. Two T2DM patients (1 GLA-treated and 1 NPH-treated) had isolated dose increases; HbA_{1c} decreased from baseline in both. No correlation was seen between changes in Ab levels and hypersensitivity.

Conclusion: Treatment with GLA is associated with generally low rates of Ab formation, without evidence of any clinical consequences. As some regulatory agencies require assessment of immunogenicity for biologics, including biosimilars, these data may provide a potential benchmark for consideration of emerging data from GLA biosimilar studies.

Study	Duration (Weeks)	GLA		NPH		Adjusted Mean Difference (SE) ^a	p-Value
		N	Mean (SD)	N	Mean (SD)		
T1DM IGAb							
3001	28	284	-1.12 (0.51)	276	-0.45 (0.51)	-0.67 (0.67)	0.3157
3101	28	138	^b	144		^b	0.0608
3004	28	254	-3.28 (0.54)	257	-0.61 (0.54)	-2.67 (0.76)	0.0005
3005	16	293	-1.35 (0.42)	296	0.83 (0.42)	-2.17 (0.58)	0.0002
T1DM HIAb							
3001	28	284	-1.55 (0.51)	276	-0.70 (0.51)	-0.86 (0.67)	0.2023
3101	28	138	^b	144		^b	NS
3004	28	254	-3.47 (0.54)	257	-0.63 (0.54)	-2.84 (0.75)	0.0002
3005	16	293	-2.56 (0.43)	296	-0.08 (0.43)	-2.48 (0.59)	0.0001
T2DM IGAb							
3002, overall	52	277	0.91 (0.69)	267	5.92 (0.70)	-5.01 (0.89)	0.0001
Insulin naïve		212	2.18 (0.79)	196	6.96 (0.82)	-4.79 (1.00)	0.0001
Insulin pretreated		65	-1.96 (1.52)	71	3.80 (1.45)	-5.75 (1.94)	0.0036
3102, insulin-naïve	28	159	^b	159		^b	0.3771
3006, insulin pretreated	28	236	-1.59 (0.44)	247	0.67 (0.42)	-2.27 (0.59)	0.0002
T2DM HIAb							
3002, overall	52	277	0.45 (0.72)	267	6.32 (0.73)	-5.87 (0.93)	0.0001
Insulin naïve		212	1.81 (0.81)	196	7.40 (0.84)	-5.60 (1.03)	0.0001
Insulin pretreated		65	-2.41 (1.65)	71	4.10 (1.58)	-6.51 (2.11)	0.0025
3102, insulin-naïve	28	159	^b	159		^b	0.4888
3006, insulin pretreated	28	237	-2.12 (0.46)	247	0.52 (0.45)	-2.64 (0.62)	0.0001

Table. Adjusted Change From Baseline to Endpoint in IGAb and HIAb Levels

^aChanges from baseline in Ab levels were analysed using a general linear model with treatment and pooled study centre as effects, and baseline value as covariate. Difference = Insulin glargine – NPH insulin.

^bFull data unavailable

SE, standard error.

Table. Adjusted Change From Baseline to Endpoint in IGAb and HIAb Levels

^aChanges from baseline in Ab levels were analysed using a general linear model with treatment and pooled study centre as effects, and baseline value as covariate. Difference = insulin glargine – NPH insulin.

^bFull data unavailable
SE, standard error.

Supported by: Sanofi US, Inc.

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Evaluation of immunogenicity of LY2963016 insulin glargine compared with insulin glargine in patients with type 2 diabetes mellitus

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Background and aims: LY2963016 (LY IGLar) is an insulin glargine product, with an identical amino acid sequence to insulin glargine (Sanofi-Aventis; IGLar), which demonstrates similar pharmacokinetic and pharmacodynamic profiles and no clinically meaningful differences from IGLar in safety or efficacy in patients with type 2 diabetes mellitus (T2DM). When evaluating similar biological products, assessment of immunogenicity is important to examine potential differences between products in the incidence and severity of human immune responses and implications this might have for safety or efficacy.

Materials and methods: This was a 24-week, Phase 3, randomised, double-blind, parallel study to compare the efficacy and safety of LY IGLar (N=376) and IGLar (N=380) in patients with T2DM on ≥ 2 oral antihyperglycaemic medications (OAMs). Patients were insulin-naïve ($HbA_{1c} \geq 7.0\%$ to $\leq 11.0\%$) or previously on IGLar ($HbA_{1c} \leq 11.0\%$). Anti-insulin glargine antibodies (% binding) were measured for the overall 24-week treatment period. The relationships between anti-insulin glargine antibody levels or treatment emergent antibody responses (TEAR) and selected efficacy and safety measures were evaluated using ANCOVA.

Results: Approximately 40% of patients (LY IGLar, 41%; IGLar, 38%) entered the study on IGLar and the remainder (LY IGLar, 59%; IGLar, 62%) were insulin-naïve. Similar proportions of patients completed the 24-week treatment period (LY IGLar, 89%; IGLar 86%). As shown in Table 1, proportions (%) of patients with detectable antibodies at baseline and throughout the treatment period were similar for LY IGLar vs IGLar ($p>.05$). There were no differences in TEAR incidence between patients randomised to LY IGLar vs IGLar and clinical outcomes (HbA_{1c} , basal insulin dose and total hypoglycaemia) were not affected by TEAR status (i.e., $p>.05$ for treatment-by-TEAR interaction for these outcomes) (Table 1). The incidence of treatment-emergent allergic events did not differ between LY IGLar and IGLar ($p>.05$) (Table 1). Clinical outcomes were also not affected by insulin antibody levels ($p>.05$).

Conclusion: These clinical safety data demonstrate a highly similar immunogenicity profile of LY IGLar to IGLar, with no effects of anti-insulin glargine antibodies on selected efficacy and safety outcomes in patients with T2DM.

Table 1 Incidence and Effect of Anti-insulin Glargine Antibodies on Clinical Outcomes

		LY IGLar N=376 ^a	IGlar N=380 ^a	p-value
Patients with Detectable Antibodies: n (%)	Baseline	20 (5.5)	13 (3.6)	.285
	Overall 24 Weeks	56 (15.3)	40 (11.0)	.100
TEAR: n (%)	Baseline	14 (3.8)	14 (3.8)	>.999
	Overall 24 Weeks	10 (4.7)	13 (5.8)	.612
Treatment Emergent Allergic Events: n (%)		21 (5.6)	27 (7.1)	.456
		LY IGLar TEAR/no TEAR N=14 ^a / N=351 ^a	IGlar TEAR/no TEAR N=14 ^a / N=351 ^a	
Effect of Overall 24 Week TEAR status on change from baseline in clinical outcomes (LSM change)	HbA _{1c} (%)	-1.21 / -1.27	-1.69 / -1.31	.185
	Basal Insulin Dose (U/Day)	39.54 / 31.75	49.63 / 31.48	.426
	Basal Insulin Dose (U/kg/Day)	0.47 / 0.35	0.57 / 0.36	.457
	Total Hypoglycaemia Rate (Episodes per 30 Days)	1.36 / 0.57	1.18 / 0.98	.676

^aN numbers reflect maximum sample size; actual sample size may vary slightly from variable to variable due to missing values LSM, least squares mean; TEAR, treatment emergent antibody response (defined as patients who were antibody negative at baseline and developed antibody binding values $\geq 1.26\%$ postbaseline, or patients with detectable antibody levels at baseline with at least a 1% increase in antibody binding value and which is 30% greater than baseline)

Clinical Trial Registration Number: NCT01421459

Supported by: Eli Lilly and Company

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Demonstration of differential immune modulation by originator and biological copies of insulin glargine in an in vitro model of human immunity

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Background and aims: Because the manufacture of insulin analogs require sophisticated production and purification procedures, it is possible originator and biological copies of insulin might have varied immunogenicity profiles based on differences in the structure (protein aggregation, denaturation), purity (contamination with endotoxins or others), and/or other characteristics of these products. To date, few studies have addressed the specific pathways of potential immune modulation triggered by biological copies of insulin glargine

Materials and methods: Here, we used an in vitro model of immunity, termed the MIMIC[®] system, to study antigen presenting cell (APC) activation and cytokine secretion induced by a range of doses of originator and biological copies of insulin glargine that are available in some countries

Results: By flow cytometric phenotype analysis, the originator and biological copy products triggered minimal changes in expression of activation markers on MIMIC[®] system-derived APCs. In contrast, some lots of the biological copies of insulin glargine, but not the originator product, were capable of inducing the secretion of some pro-inflammatory markers, such as IL-8, in the treated MIMIC[®] cultures. These immunogenicity differences appear to be due to the effect of the compounds on monocytic and/or lymphocytic populations in the in vitro human cell system. Of note, all insulin analogs included in this analysis were comparable in their insulin receptor activity in vitro and typically generated similar analytical profiles.

Conclusion: In contrast to the originator product, some of the biological copies of insulin glargine (variable by lot) triggered the induction of pro-inflammatory cytokines in the MIMIC[®] system, which might indicate a difference in the biochemical composition of some of these insulin biological copies. While the mechanism and differences causing these effects is still under investigation, and the potential clinical impact is unknown, the data presented here suggests there might be different immune modulation potentials between originator and copy insulin compounds. Further investigation of the potential clinical relevance and/or mechanisms regulating this response may be warranted.

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Binding kinetics, internalisation and localisation of long-acting basal insulin peglispro (BIL) and insulin receptor in cells

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Background and aims: BIL (LY2605541) is a novel, PEGylated insulin lispro that has a large hydrodynamic size. It has a prolonged duration of action which is related to a delay in insulin absorption and a reduction in clearance. We compared the binding kinetics, intracellular localization, in vitro clearance, and effect on activation and localization of human insulin receptor (hIR) of BIL with those of insulin lispro and biosynthetic human insulin (BHI).

Materials and methods: Cellular internalization and localization of BIL, BHI, insulin lispro, and tyrosine-phosphorylated hIR (hIR-pY) were observed by immunofluorescent confocal imaging in U2OS cells that overexpress hIR. Binding and kinetic properties of BIL and BHI were measured in IM9 cells using radio-ligand binding techniques. The in vitro clearances of BIL and BHI were studied in HEK cells overexpressing hIR.

Results: Co-localization studies using antibodies to insulin or PEG, and the early endosomal marker, EEA1, showed that the internalization and subcellular localization pattern of BIL were similar to those of BHI and insulin lispro; all were rapidly internalized and co-localized with EEA1. During ligand washout, concomitant loss of insulin, PEG methoxy group, and PEG polymer backbone immunostaining was observed for BIL similar to the loss of insulin immunostaining observed for insulin lispro and BHI. Co-localization studies using an antibody to the lysosomal marker LAMP2 did not reveal evidence of lysosomal localization for insulin lispro, BHI, or BIL, using either insulin or PEG immunostaining reagents. In vitro clearance studies showed efficient insulin receptor-mediated clearance of BHI and the insulin portion of BIL. In contrast, the concentration of PEG polymer backbone appeared to decrease significantly more slowly. This is consistent with a process whereby, upon internalization of BIL, the insulin moiety is degraded, whereas the PEG backbone is recycled out of cells, and is consistent with the above observation that no PEG was detected in lysosomes. BIL and BHI both induced rapid phosphorylation and internalization of hIR. Co-staining of hIR-pY and EEA1 showed that hIR promptly entered early endosomes after BIL and BHI treatment. Minimal lysosomal localization of hIR-pY was detected with either ligand. Ligand washout studies suggested a more rapid dephosphorylation of hIR-pY after treatment with BIL than with BHI. Whole cell binding analysis performed with IM9 cells also showed slightly faster hIR dissociation kinetics for BIL than for BHI. The pH dependence of the dissociation rate and the magnitude of accelerated dissociation from the receptor at high concentrations of BIL were similar to those of BHI. Affinity of BIL for hIR was ~20-fold lower than that of BHI.

Conclusion: The mechanism of insulin receptor activation by BIL is similar to that of BHI as evidenced by preservation of pH and ligand concentration dependences of the dissociation rate. The reduction of affinity to the insulin receptor is attributable mainly to a decrease in the association rate constant. The insulin and PEG moieties of BIL undergo a dynamic cellular process of rapid internalization and transport to early endosomes after stimulation of hIR-pY followed by loss of cellular immunostaining in a manner similar to those of insulin lispro and BHI suggestive of efficient transport of the PEG backbone out of cells.

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Pharmacological evaluation of once-weekly potentials by combination of long-acting insulin with long-acting exendin4 in an animal model

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Background and aims: Combination of basal insulin and GLP-1 receptor agonist could provide diabetic patients with improved efficacy and safety. However, the recently developed combination products require an once-daily administration, which is still frequent and thus negatively affects treatment adherence and efficacy. We have developed the novel long-acting basal insulin analog and exendin-4 analog, namely HM12470 (once-weekly regimen) and HM11260C (once-weekly or monthly regimen), respectively, using

LAPSCOVERY technology. Because of their prolonged and flatness pharmacokinetic (PK) / pharmacodynamic (PD) properties, the combination of HM12470 and HM11260C could maximize the dosing convenience as well as the therapeutic advantages. The objective of this study was to evaluate the once-weekly potentials of HM12470 and HM11260C combination in animal model.

Materials and methods: Human PK profile of HM12470 was predicted based on PK results of three different animal species using Wajima C_{ss} -MRT method and compared with that of HM11260C in human. *db/db* mice were chronically administrated with HM12470 and/or HM11260C. To evaluate the glucose lowering efficacy, 4h fasting blood glucose (FBG) levels were measured every 3–4 days. Body weight was determined every 2 days for proper drug administration. At the end of study, HbA_{1c} levels were determined. To evaluate the PK profiles of combination treatment, serum concentration of HM12470 and/or HM11260C were quantified after single administration of HM12470 and/or HM11260C in SD rats.

Results: The half-life of HM12470 in human was predicted as 132hr which was similar with HM11260C (140hr), suggesting their once-weekly combination potentials. In *db/db* mice, once-weekly mimetic administration (Q2D) of HM12470 and HM11260C showed significantly reduced FBG levels compared to that of individual compounds. Consistent with FBG control, HM12470 and HM11260C combination group had lower HbA_{1c} level ($6.8 \pm 0.8\%$) than HM12470 ($9.0 \pm 0.6\%$) or HM11260C ($8.0 \pm 1.7\%$) groups, indicating improved glycemic control by combination treatment. Moreover, this improved glycemic control was maintained even by reducing HM12470 dose in combination treated group. Furthermore, combination treatment attenuated body weight gain observed in HM12470 only groups. As to the pharmacokinetic profiles, administration of HM12470 and HM11260C did not affect pharmacokinetic profiles of either individual compounds in SD rats, ruling out possible drug-drug interference.

Conclusion: Based on PK prediction results, HM12470 could be a best partner of HM11260C for the once-weekly combination therapy. Of note, combination of HM12470 and HM11260C not only improved glycemic control, but also controlled unwanted body weight gain by insulin drug in *db/db* mice. Given that there were no PK interference between HM12470 and HM11260C, our results collectively demonstrate that combination of HM12470 and HM11260C provide optimal convenience as well as therapeutic advantages, shedding light on ideal therapy for the management of diabetic patients.

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Hypoglycaemia in people with type 2 diabetes achieving vs not achieving HbA_{1c} target levels over 4 years in routine clinical practice:

analysis of the CREDIT study

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Background and aims: To evaluate the incidence and rate of hypoglycaemia in people with type 2 diabetes achieving vs not achieving HbA_{1c} < 7.0 % (53 mmol/mol) over 4 years after starting insulin therapy in routine clinical practice in Europe, Canada, and Japan.

Materials and methods: CREDIT (Cardiovascular Risk Evaluation in people with Type 2 Diabetes on Insulin Therapy) was a noninterventional study designed to examine the relationship between HbA_{1c} and cardiovascular events and to provide insight into current, real-world practices of the use of insulin by 2999 people beginning insulin, with up to 54 months' follow up (n = 2272 with 4-year data). For this analysis, records of symptomatic, severe, and nocturnal symptomatic hypoglycaemia were collected for the 6 months prior to the 1, 2, 3, and 4-year follow-up intervals. Updated mean HbA_{1c} was calculated as the average of normalized HbA_{1c} measurements from 1 month after insulin initiation up to follow-up year values. Hypoglycaemia events were categorised by whether or not the person experiencing the event had updated mean HbA_{1c} < 7.0 % or not at the follow-up visit closest to the event.

Results: Overall mean (SD) HbA_{1c} declined from 80 (21) mmol/mol (9.5 [2.0] %) when starting insulin to 60 (14) mmol/mol (7.6 [1.3] %) at 4 years (median 56 mmol/mol [7.3 %]). During the last 6 months of year 1, 2, 3, and 4 follow-up, the proportion of people experiencing ≥ 1 hypoglycaemic event was higher for those who achieved target (range 17.7%–24.6%) than for those who did not (range: 15.8%–16.8%) (Table). This was also true for incidence rate of any documented symptomatic hypoglycaemia (1.18–1.40 vs 0.75–0.96 events/person), and nocturnal symptomatic hypoglycaemia (0.14–0.24 vs 0.12–0.20 events/person). The percentage of people who experienced ≥ 1 severe hypoglycaemic event for the 4 half-yearly follow-up periods was also higher for those to target (range 3.0%–4.6%) than for those not (0.9%–1.6%). Mean rates of severe hypoglycaemia were 0.04–0.20 vs 0.02–0.04 events/person, while severe nocturnal hypoglycaemia was 0.02–0.06 vs 0.00–0.01 events/person.

Conclusion: People with type 2 diabetes starting insulin who achieve updated mean target HbA_{1c} levels in routine clinical practice do experience higher rates of symptomatic and nocturnal hypoglycaemia than those failing to achieve target HbA_{1c}. However, the difference between groups in the percentage of individuals with hypoglycaemia seems to diminish over time, and rates for both groups are relatively modest. Moreover, the rate of severe hypoglycaemia is low and could not be distinguished by whether target HbA_{1c} is achieved. Initiation of insulin in the CREDIT population was associated

with a significant improvement in glycaemia. These hypoglycaemia data are consistent with a positive benefit risk ratio for this therapeutic strategy.

Table: Hypoglycaemia in the last 6 months in people achieving and not achieving updated mean HbA_{1c} of < 7.0 % (target)

	Year 1	Year 2	Year 3	Year 4
Participants				
To target, n	710	685	621	586
Not to target, n	1919	1842	1741	1602
Symptomatic hypoglycaemia				
Anytime, people, n (%)				
To target	175 (24.6)	155 (22.7)	110 (17.7)	107 (18.3)
Not to target	315 (16.5)	309 (16.8)	274 (15.8)	259 (16.2)
Anytime, events/person				
To target	1.40 ± 4.79	1.35 ± 4.34	1.18 ± 4.49	1.39 ± 4.76
Not to target	0.96 ± 4.65	0.93 ± 4.97	0.75 ± 2.59	0.92 ± 3.58
Nocturnal, events/person				
To target	0.19 ± 1.23	0.17 ± 0.78	0.14 ± 0.77	0.24 ± 1.16
Not to target	0.13 ± 0.82	0.12 ± 0.92	0.13 ± 0.79	0.20 ± 1.20
Severe hypoglycaemia				
Anytime, people, n (%)				
To target	21 (3.0)	25 (3.6)	21 (3.4)	27 (4.6)
Not to target	29 (1.5)	17 (0.9)	28 (1.6)	17 (1.1)
Anytime, events/person				
To target	0.04 ± 0.26	0.08 ± 0.50	0.10 ± 0.66	0.20 ± 1.17
Not to target	0.03 ± 0.44	0.02 ± 0.23	0.04 ± 0.45	0.04 ± 0.61
Nocturnal, events/person				
To target	0.02 ± 0.15	0.03 ± 0.25	0.02 ± 0.15	0.06 ± 0.47
Not to target	0.01 ± 0.35	0.00 ± 0.04	0.01 ± 0.14	0.01 ± 0.10

Data are presented as n (%) or mean ± SD.

Supported by: Sanofi

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IDegAsp lowers the rate of hypoglycaemia vs biphasic insulin aspart 30 in adults with type 2 diabetes achieving glycaemic target (HbA_{1c} <7.0%): a meta-analysis

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Background and aims: Insulin degludec/insulin aspart (IDegAsp) is a soluble co-formulation of 70% insulin degludec and 30% insulin aspart. The objective of this post-hoc meta-analysis was to compare hypoglycaemia rates for IDegAsp, vs. biphasic insulin aspart 30 (BIAsp 30) in adults with type 2 diabetes (T2D) achieving the ADA/EASD target of HbA_{1c} <7% at end of trial (EOT).

Materials and methods: This analysis included two 26-week trials (NN5401-3592/97) comparing twice-daily IDegAsp vs BIAsp 30. HbA_{1c}, fasting plasma glucose (FPG) and insulin dose were analysed using an ANCOVA model; episodes of hypoglycaemia were analysed using a negative binomial regression model. Confirmed hypoglycaemia was defined as self-reported PG <3.1 mmol/L or severe (requiring third-party assistance); nocturnal confirmed hypoglycaemia had onset 00:01–05:59 am, inclusive.

Results: In total, 49.1% (426/868) of subjects achieved EOT HbA_{1c} of <7% (IDegAsp: 49.2% [248/504]; BIAsp 30: 48.9% [178/364]) and were included in the meta-analysis. Treatment groups had similar EOT HbA_{1c} levels (6.4% vs. 6.4%; estimated treatment difference [ETD]: 0.00% [-0.07; 0.07], p=0.996), whereas IDegAsp achieved a significantly greater reduction from baseline in FPG (-3.0 vs. -2.1 mmol/L; ETD: -1.1 mmol/L [-1.4; -0.9] p<0.0001). Mean daily insulin dose at EOT was significantly lower for IDegAsp than BIAsp 30 (0.9 vs. 1.1 U/kg; estimated dose ratio: 0.77 [0.72; 0.84]; p<0.0001). IDegAsp was associated with a significantly lower rate of overall confirmed hypoglycaemia (rate ratio [RR] IDegAsp/BIAsp 30: 0.70 [0.55; 0.90], p=0.0047) and a significantly lower rate of nocturnal hypoglycaemia than BIAsp 30 (RR: 0.34 [0.22; 0.51], p<0.0001). Severe hypoglycaemia rates were low and similar between groups.

Conclusion: The significant difference vs. BIAsp 30 in overall and nocturnal confirmed hypoglycaemia in adults achieving target glycaemia (HbA_{1c} <7%) support the flat and stable pharmacodynamic profile of the basal degludec component in IDegAsp.

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New insulin glargine 300 U/mL: glycaemic control and hypoglycaemia in Japanese people with type 1 diabetes mellitus (EDITION JP 1)

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Background and aims: EDITION JP 1 studied the efficacy and safety of new insulin glargine 300 U/mL (Gla-300) versus glargine 100 U/mL (Gla-100) in Japanese people with T1DM using basal plus mealtime insulin.

Materials and methods: In the 6-month, multicentre, open-label, phase 3 trial, participants (n=243; mean age 45.2 years; T1DM duration 13.0 years; HbA_{1c} 8.1 %) were randomised to once-daily Gla-300 or Gla-100 in combination with mealtime insulin. Basal insulin was titrated to target FPG 4.4–7.2 mmol/L (80 to 130 mg/dL). Primary endpoint was HbA_{1c} change from baseline to month 6.

Results: Similar HbA_{1c} decreases were seen with both Gla-300 and Gla-100 (LS mean [SE] -0.30 [0.06] % and -0.43 [0.06] %; LS mean difference 0.13 [95% CI: -0.03 to 0.29] %). Fewer participants who received Gla-300 experienced confirmed or severe nocturnal hypoglycaemic events versus Gla-100 over the 6-month study period, with the greatest difference observed during the first 8 weeks (Table). The rate of hypoglycaemic events per participant-year at any time of the day (24 hours) was lower with Gla-300 than Gla-100. Severe hypoglycaemia was infrequent in either group. Comparable numbers of adverse events were recorded in each group.

Conclusion: In Japanese people with T1DM using basal and mealtime insulin, Gla-300 provides comparable effective glycaemic control with less nocturnal hypoglycaemia, particularly in the first 8 weeks, and no increase in daytime hypoglycaemia versus Gla-100.

Table: Confirmed or severe hypoglycaemia in the EDITION JP 1 study (safety population)

		Nocturnal hypoglycaemia (00:00–05:59)		Hypoglycaemia at any time of day (24 h)	
		Gla-300 (N=122)	Gla-100 (N=121)	Gla-300 (N=122)	Gla-100 (N=121)
Confirmed (≤3.9 mmol/L [≤70 mg/dL]) or severe hypoglycaemia					
Baseline to month 6	% people ≥1 event	68.9	81.0	96.7	97.5
	RR (95% CI)	0.85 (0.73 to 0.99)		0.99 (0.95 to 1.04)	
	Rate per participant-year	7.46	11.24	75.31	94.76
Baseline to week 8	% people ≥1 event	43.4	61.2	86.9	95.0
	RR (95% CI)	0.71 (0.56 to 0.91)		0.91 (0.84 to 0.99)	
	Rate per participant-year	7.48	12.79	82.77	119.10
Week 9 to month 6	% people ≥1 event	61.7*	73.7†	94.2*	93.2†
	RR (95% CI)	0.84 (0.70 to 1.00)		1.01 (0.95 to 1.08)	
	Rate per participant-year	7.45	10.53	71.86	83.58
Confirmed (<3.0 mmol/L [≤54 mg/dL]) or severe hypoglycaemia					
Baseline to month 6	% people ≥1 event	36.9	53.7	78.7	90.9
	RR (95% CI)	0.69 (0.52 to 0.91)		0.87 (0.78 to 0.96)	
	Rate per participant-year	2.00	4.07	18.91	23.28
Baseline to week 8	% people ≥1 event	22.1	37.2	59.0	80.2
	RR (95% CI)	0.60 (0.40 to 0.89)		0.74 (0.62 to 0.87)	
	Rate per participant-year	2.12	5.72	21.95	30.33
Week 9 to month 6	% people ≥1 event	27.5*	44.9†	71.7*	85.6†
	RR (95% CI)	0.61 (0.43 to 0.87)		0.84 (0.73 to 0.96)	
	Rate per participant-year	1.94	3.31	17.50	20.02

CI, confidence interval; RR, relative risk. *N=120; †N=118

Clinical Trial Registration Number: NCT01689129

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Glycaemic control and hypoglycaemia in Japanese people with type 2 diabetes mellitus receiving new insulin glargine 300 U/mL in combination with OADs (EDITION JP 2)

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Background and aims: EDITION JP 2 studied the efficacy and safety of new insulin glargine 300 U/mL (Gla-300) versus glargine 100 U/mL (Gla-100) in Japanese people with T2DM using basal insulin plus OAD(s).

Materials and methods: In this 6-month, multicentre, open-label, phase 3 study, participants (n=241; mean age 60.8 years; mean BMI 25.3 kg/m²; mean duration of T2DM 14.0 years; mean HbA_{1c} 8.0 %) were randomised to receive once-daily Gla-300 or Gla-100 plus OAD(s). Insulin was titrated to target FPG 4.4–5.6 mmol/L (80–100 mg/dL). The primary endpoint was HbA_{1c} change from baseline to month 6.

Results: HbA_{1c} decreased similarly in both groups (LS mean [SE] −0.45 [0.06] % for Gla-300 and −0.55 [0.06] % for Gla-100; LS mean difference 0.10 [95% CI: −0.08 to 0.27] %). Fewer participants experienced any hypoglycaemic events during 6 months with Gla-300 versus Gla-100. The number of participants with ≥1 confirmed (≤3.9 mmol/L) or severe hypoglycaemic event (24 h and nocturnal) was consistently lower with Gla-300 compared with Gla-100, and rate per participant-year was lower with Gla-300 versus Gla-100 (Table). Severe hypoglycaemia was infrequent in both groups. LS mean (SE) weight change was −0.62 (0.19) kg for Gla-300 and 0.37 (0.19) kg for Gla-100. Similar safety profiles were observed in both groups.

Conclusion: In Japanese people with T2DM using basal insulin plus OAD(s), Gla-300 provides comparable effective glycaemic control with fewer hypoglycaemic events, particularly during the first 8 weeks.

Table: Hypoglycaemia in the EDITION JP 2 study (safety population)

		Nocturnal Hypoglycaemia (00:00–05:59)		Hypoglycaemia at any time of day (24 h)	
		Gla-300 (N=120)	Gla-100 (N=120)	Gla-300 (N=120)	Gla-100 (N=120)
Confirmed (≤3.9 mmol/L [≤70 mg/dL]) or severe hypoglycaemia					
Baseline to month 6	% people ≥1 event	28.3	45.8	65.0	76.7
	RR (95% CI)	0.62 (0.44 to 0.88)		0.86 (0.73 to 1.01)	
	Rate per participant-year	2.18	4.98	10.48	16.52
Baseline to week 8	% people ≥1 event	13.3	16.7	37.5	55.0
	RR (95% CI)	0.83 (0.45 to 1.52)		0.69 (0.52 to 0.91)	
	Rate per participant-year	2.25	2.67	9.00	15.86
Week 9 to month 6	% people ≥1 event	25.4*	43.7†	60.2*	72.3†
	RR (95% CI)	0.58 (0.40 to 0.85)		0.84 (0.70 to 1.01)	
	Rate per participant-year	2.15	6.03	11.17	16.91
Confirmed (<3.0 mmol/L [<54 mg/dL]) or severe hypoglycaemia					
Baseline to month 6	% people ≥1 event	10.0	10.8	16.7	19.2
	RR (95% CI)	0.94 (0.44 to 2.00)		0.86 (0.50 to 1.48)	
	Rate per participant-year	0.24	0.35	0.61	0.64
Baseline to week 8	% people ≥1 event	3.3	5.8	6.7	10.0
	RR (95% CI)	0.61 (0.19 to 1.94)		0.66 (0.28 to 1.55)	
	Rate per participant-year	0.21	0.53	0.48	0.86
Week 9 to month 6	% people ≥1 event	6.8*	6.7†	11.9*	12.6†
	RR (95% CI)	1.01 (0.39 to 2.62)		0.93 (0.47 to 1.83)	
	Rate per participant-year	0.25	0.27	0.67	0.54

CI, confidence interval; RR, relative risk. *N=118; †N=119

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The beneficial effect of insulin degludec on nocturnal hypoglycaemia and insulin dose in type 1 diabetic patients: a systematic review and meta-analysis of randomised trials

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Background and aims: Insulin degludec is a new-generation ultra-long acting basal insulin which offers a significantly more predictable glucose-lowering effect than other long-acting insulin analogues. To compare the effect of treatment with insulin degludec and long-acting insulin analogues glargine and detemir in type 1 diabetic (T1D) patients by means of a systematic review and meta-analysis.

Materials and methods: The following electronic databases were searched up to January 2014: MEDLINE, EMBASE, The Cochrane Library. Additional references were obtained from the reviewed articles. There were included randomized controlled trials of at least 12 weeks' duration with basal-bolus regimen therapies in T1D patients.

Results: Current analysis included 4 studies involving 1,846 T1D patients. The combined data from all trials showed a statistically significant reduction in the basal insulin dose (MD 0.042, 95% CI −0.067 to −0.018, p=0.001) and the total daily insulin dose (MD −0.072, 95% CI 0.016 to −0.027, p=0.002) in the degludec group compared to other long-acting analogues. There was also a significant reduction of nocturnal hypoglycemia in the degludec group compared to the controls (Rate Ratio 0.697, 95% CI 0.617 to 0.786, p=0.000). There were no differences between the groups in terms of HbA_{1c} values, fasting plasma glucose (FPG), adverse events.

Conclusion: Basal-bolus treatment with insulin degludec was superior to long-acting insulin analogues detemir and glargine in reducing the rate of nocturnal hypoglycemia. In comparison to other long-acting analogues, treatment with insulin degludec was safe, patients obtained similar metabolic control expressed by HbA_{1c} and FPG levels with the added benefit of a reduced basal and total insulin dose.

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Hypo-/hyperglycaemia alert based on online glucose prediction using a global model for type 1 diabetes

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Background and aims: Avoiding hypo/hyperglycemia is a major challenge for diabetes mellitus. A timely alert of hypo/hyperglycemia episodes before they occur can allow enough time for the patient to take necessary actions to avoid hypo/hyperglycemia.

Materials and methods: A global model, which is developed based on one subject and then applied to new subjects without any customization, is used for early hypo/hyperglycemia alert based on online glucose prediction using clinical data provided by BD. Two autoregressive (AR) modeling methods are considered using standard least squares algebra: (i) global prediction model; (ii) subject-dependent model. Both are developed based on time-series glucose data with 2 min as sampling interval. Also, to evaluate the role of high-frequency glucose data, the glucose data are filtered by a first-order low-pass filter with 30min the as threshold. Online short-term glucose predictions are made for 15 subjects for alert of hyper/hypoglycemia based on filtered and non-filtered data.

Results: The results are summarized in Table 1 as evaluated by time lag which is the time difference between the predicted hyper/hypoglycemia and the measured event.

Conclusion: The results indicate that the accuracy of global model is comparable to or even better than that of subject-dependent model for alert of hyper/hypoglycemia based on the index of time lag. However, the sensitivity for hypos using global model is lower than that using subject-dependent model. Also, the model developed based on filtered glucose data can get smaller time

lag of hyper/hypoglycemia alert than that based on raw glucose data since the high-frequency noises are removed after low-pass filtering.

Table 1 Hyper/hypoglycemia alert accuracy for 15 subjects and 30 minute predictions (mean \pm standard deviation)

Type of Model	Time Lag for hyper event (samples)	Sensitivity for hyper event (%)	Specificity for hyper event (%)	Time Lag for hypo event (samples)	Sensitivity for hypo event (%)	Specificity for hypo event (%)
non-filtered	Global model	11.86 \pm 5.75	62.86	100	0.0	12.50
	Subject-dependent model	11.64 \pm 5.01	71.43	92.86	7.25 \pm 8.38	28.57
filtered	Global model	11.41 \pm 5.12	82.15	100	7.50 \pm 8.66	25.00
	Subject-dependent model	11.37 \pm 5.09	77.14	92.86	6.88 \pm 7.45	50.00

The global model is developed based on subject #6; similar results are obtained for global model developed based on other subjects.

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979

Assessment of glycaemic control and risk of hypoglycaemia for two basal-bolus algorithms in hospitalised patients with diabetes mellitus type 2

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Background and aims: Current guidelines recommend pre-meal blood glucose (BG) levels of <7.8mmol/l in the hospital. The aim of this analysis was to compare two versions of a workflow-integrated algorithm for basal-bolus insulin therapy (REACTION algorithm) for glycaemic management in patients with diabetes mellitus type 2 (T2D) hospitalised at the general ward.

Materials and methods: For both algorithms BG measurements were performed four times daily (pre-breakfast, pre-lunch, pre-dinner, bedtime); insulin injections were given according to the algorithm. Advice for total daily dose (TDD) (50% basal insulin, 50% pre-meal bolus insulin) was generated once daily. In the initial algorithm bolus insulin dose was evenly distributed over the day; in the refined algorithm bolus insulin dose was redistributed whereas TDD and the 50:50 ratio remained unchanged.

Results: 52 (15 female, age 69 \pm 11 years, HbA_{1c} 76 \pm 30mmol/mol, BMI 30 \pm 6kg/m², diabetes duration 14 \pm 11 years) and 95 (42 female, age 68 \pm 11 years, HbA_{1c} 65 \pm 21mmol/mol, BMI 31 \pm 7kg/m², diabetes duration 15 \pm 11 years) T2D patients were treated with the initial and the refined algorithm respectively. Mean BG was 8.8 \pm 1.8mmol/l (initial algorithm) vs. 8.5 \pm 1.8mmol/l (refined algorithm). The overall risk of getting a hypoglycaemic episode (<3.9 mmol/l) during the first 10 treatment days was 3% vs. 1.3% (initial vs. refined algorithm). There was no significant change in hypoglycaemia occurrence over the treatment time and no influence of time of day between groups. Mean TDD was 45 \pm 26U (basal: 22 \pm 18U, bolus: 24 \pm 16U) for the initial algorithm and 47 \pm 26U (basal: 24 \pm 18U, bolus: 24 \pm 18U) for the refined algorithm.

Conclusion: The refined version of the REACTION algorithm could establish similar glycaemic control with reduced risk of hypoglycaemia. Insulin doses were comparable for the two versions of the algorithm. The REACTION algorithm has the potential to improve glycaemic management in the hospital setting.

Clinical Trial Registration Number: NCT014072, NCT01932775, NCT01766752

Supported by: European Commission, Project REACTION (FP7 248590)

980

Sustained glycaemic control and less hypoglycaemia with new insulin glargine 300 U/ml vs 100 U/ml: 1-year results in type 2 diabetes with basal and mealtime insulin (EDITION 1)

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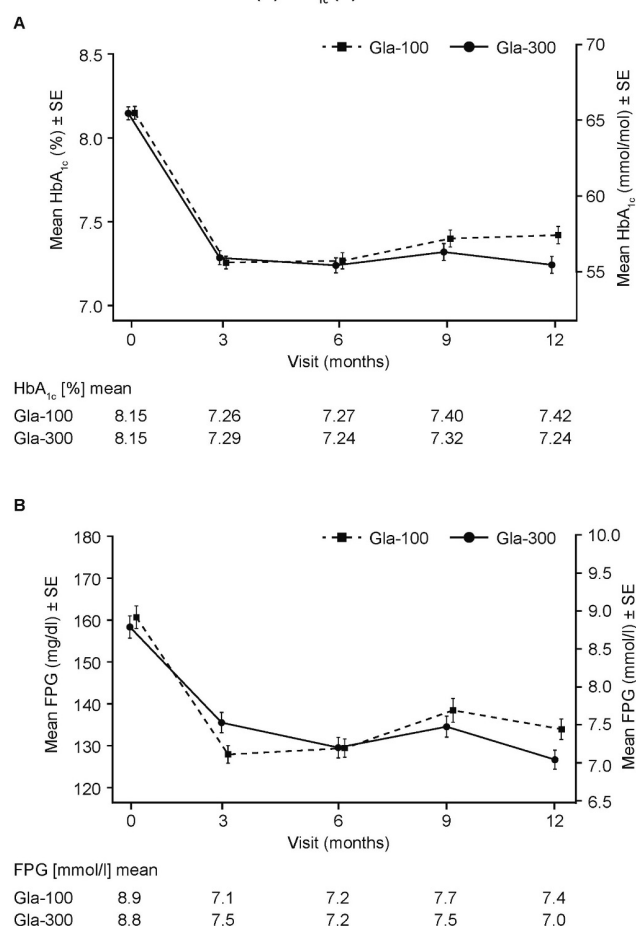
Background and aims: EDITION 1 studied the efficacy and safety of new insulin glargine 300 U/ml (Gla-300) versus glargine 100 U/ml (Gla-100) in people with type 2 diabetes mellitus using basal plus mealtime insulin.

Materials and methods: In EDITION 1, 807 people with elevated HbA_{1c} were randomised to titrate Gla-300 or Gla-100 once daily in the evening for 6 months, continuing the mealtime insulin. In a 6-month open-label extension, participants continued Gla-300 or Gla-100; 89% and 88% completed 12 months of treatment.

Results: Improved glycaemic control was maintained over 12 months in both groups (LS mean difference Gla-300 vs Gla-100: -0.17 [95% CI: -0.30 to -0.05] % for HbA_{1c} and -0.34 [95% CI: -0.69 to 0.01] mmol/l for FPG) (Figure). Basal insulin doses were higher with Gla-300 than Gla-100 after 12 months (1.03 vs 0.90 U/kg). During the 12 months of treatment, a similar percentage of participants had ≥ 1 confirmed (≤ 3.9 mmol/l [≤ 70 mg/dl]) or severe hypoglycaemic event at any time of the day (85.9% with Gla-300 vs 91.5% with Gla-100; RR 0.94 [95% CI: 0.89 to 0.99]). During the night this percentage was lower in Gla-300 (54.5% vs 64.7% in Gla-100; RR 0.84 [95% CI: 0.75 to 0.94]). Severe hypoglycaemia was reported by 6.7% of Gla-300- and 7.5% of Gla-100-treated participants. No between-treatment differences in adverse events were seen.

Conclusion: In conclusion, over 1 year of treatment in people with type 2 diabetes mellitus using basal and mealtime insulin, Gla-300 provided sustained glycaemic control with a lower incidence of hypoglycaemia compared with Gla-100.

Figure: Glycemic control over 12 months in EDITION 1 study.
(A) HbA_{1c} (B) FPG



Clinical Trial Registration Number: NCT01499082

Supported by: Sanofi

PS 080 Gastrointestinal liners and e-health opportunities

981

Acute and sub-acute effects of the endobarrier on glucose homeostasis and appetite in obese uncontrolled type 2 diabetes mellitus patients

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Background and aims: Diabetes is a prevalent metabolic disease accompanied by an increased risk for cardiovascular disease as well as micro-vascular complications. To date, bariatric surgery has become one of the most effective means of providing durable, clinically-significant resolution of diabetes, with the majority of subjects with T2DM undergoing these procedures experiencing a remission of their disease. One of the theories behind the early resolution of diabetes after the Roux-en-Y Gastric bypass operation claims that the mediating factor lies within the bypassing of the duodenum by the ingested food. The Endobarrier gastrointestinal liner system (by GI Dynamics®) is an endoscopically-delivered device that mimics gastric bypass surgery by shielding the duodenum and upper jejunum from contact with chyme.

Materials and methods: An Endobarrier device was placed in the duodenum via an endoscopic procedure in 33 uncontrolled diabetic obese subjects. Acute and sub-acute effects of the device on glucose homeostasis were evaluated using a Continuous Glucose Monitoring (CGM) recording device for one week, as of two days prior to device implantation until four days after its removal. The effect of the device on appetite was evaluated using the 5-question visual analog scale, where each question was given a score between 1–10.

Results: Our population included 17 females and 16 males with mean diabetes duration of 10.4 (SD±5.8) years, most were insulin treated. Baseline HbA1c level was of 9.3% (SD±1.0) and baseline body mass index was 38.0 kg/m² (SD±5.0). CGM data revealed a significant decrease in average daily glucose levels from 243.2 mg/dL (SD±63.4) one day before the procedure to 227 mg/dL (SD±53.6) (p value = 0.002) on the day of the procedure, while the subjects were fasted most of the day. As of the first day after the procedure, despite the fact that eating was initiated, prandial medications were terminated and basal insulin dose was lowered by 50%, the recorded glucose levels continued to decrease and reached a decrease of 30.3 mg/dL (SD±69.4) (p value= 0.048) vs. baseline on the last day of CGM recording. Whereas no significant change in the VAS score was recorded after one week, one month after the Endobarrier insertion, a significant change of 1.1 points (SD±1.7) (p value=0.003) vs. baseline was recorded suggesting a significant decrease in appetite one month after the procedure, despite a weight loss of 4.2 kg at this time-point.

Conclusion: Endobarrier therapy in advanced uncontrolled obese diabetic patients significantly improves their glucose homeostasis, as early as the first day after the procedure. This improvement becomes even more prominent as days go by and eating becomes more regular. Despite a substantial weight loss, appetite is significantly suppressed one month after the insertion of the device. EndoBarrier seems to have an early lowering effect on blood glucose as well as a suppressive effect on appetite, rendering it an attractive option for the care of „diabetes“ in this challenging population.

Clinical Trial Registration Number: NCT01718457

Supported by: IASD

982

Effects of duodeno-jejunal bypass liner on bile acid homeostasis in patients with severe obesity

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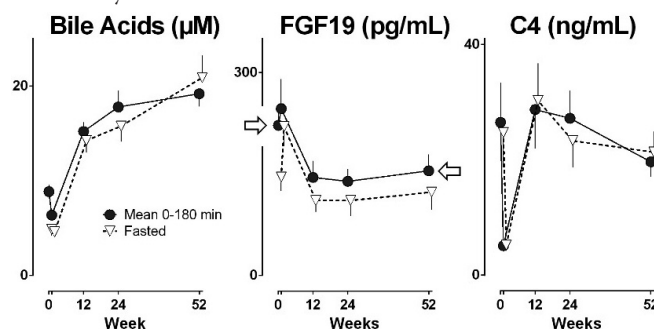
Background and aims: Endoscopic placement of a duodenojejunal bypass liner (DJBL) elicited a 16.1 ± 1.6% loss of body weight in 17 morbidly obese

patients, and normalization of HbA1c (<6.3%) in 5/7 of the subset with type 2 diabetes. DJBL excludes nutrient contact with the duodenal mucosa and thus mimics aspects of Roux-en-Y gastric bypass surgery. Biliary and pancreatic secretions pass outside the liner until they mix with chyme in the proximal jejunum. We examined the effects of DJBL on plasma concentrations of bile acids (BA) and other markers of BA metabolism.

Materials and methods: Plasma total BA concentration was measured enzymatically. Primary and secondary bile acids, their taurine and glycine conjugates, and 7α-hydroxy-cholest-4-ene-3-one (C4), an intermediate used as a correlate of BA synthetic rate, were determined by HPLC/MS in samples taken at fasting and over 3 hours after a standard (Ensure) test meal. Testing occurred before, 1, 12, 24, and 52 weeks after DJBL placement in severely obese patients (age 34.8±9.5 years; body mass index 42.6±5.2 kg/m²). FGF-19, a feedback inhibitor of BA synthesis secreted from the ileum, was measured by ELISA.

Results: One week after DJBL placement, fasting and post-meal total BA concentrations, and C4 concentrations abruptly decreased; FGF-19 concentrations increased. Thereafter, fasting and post-meal total BAs increased (1.7-fold, 2.3-fold, respectively, from baseline; each P<0.05). Changes were associated with 10-fold (fasting) and 5-fold (post-meal) increases in unconjugated primary and, respectively, 25-fold and 5-fold increases in unconjugated secondary BAs. Conjugated BAs did not change. Aside from the transient perturbations at week 1, FGF-19 progressively declined. This was especially apparent in the post-meal samples (arrows). Aside from week 1, C4 was unchanged.

Conclusion: Marked increase in circulating BAs with DJBL, attributable to unconjugated species, occurs without an apparent increase in BA synthetic rate (unchanged C4) but with a reduction in BA sensing in the ileum (reduced FGF-19). Possible mechanisms may include early bacterial deconjugation and passive absorption of secreted BAs. The contribution of these BA changes to the benefits of DJBL, and other procedures such as RYGBS, merits further study.



Clinical Trial Registration Number: NCT00985491

983

Duodenal-jejunal bypass liner (DJBL) exerts prompt and robust metabolic changes in severely obese patients

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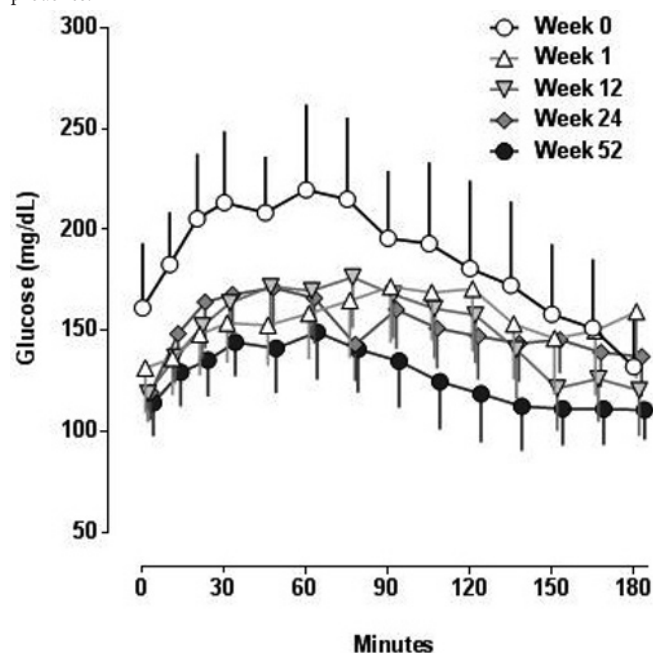
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Background and aims: The endoscopically placed duodenal-jejunal bypass liner (DJBL) prevents nutrient contact with the duodenal mucosa, thereby replicating elements of gastric bypass surgery, and eliciting robust weight loss and metabolic improvement in obese subjects with or without diabetes. We examined the time-dependent effects of DJBL on clinical, metabolic and hormonal measures out to 52 weeks in a cohort of severely obese subjects (n=17; age 34.8±9.5 years; body mass index 42.6±5.2 kg/m², 77% female) some of whom had overt type 2 diabetes (DM subset n=7; A1C 7.07±1.48%).

Materials and methods: Clinical endpoints (A1C, body weight) and mixed meal (Ensure) challenge samples were collected at baseline and 1, 12, 24, and 52 weeks for measurement of plasma glucose, FFA, triglycerides, c-peptide, glucagon, and GLP-1.

Results: In the overall cohort, there was prompt and robust weight loss observed that plateaued at 6–12 months (weight loss % at 52 weeks: overall -15.9%, non-DM -14.3%, DM -18.2%). A1C in the DM group decreased to $6.24 \pm 1.18\%$ at 52 weeks. Lowering of both fasting and post-prandial glucose (see figure) in the DM group was observed 1 week post implant and was maintained to 52 weeks. GLP-1 responses were augmented and triglycerides and FFAs suppressed during meal challenges from 12 to 52 weeks ($P < 0.05$ vs. baseline for AUC for all analytes). In addition, augmented c-peptide and suppressed glucagon was observed during meal challenge in the DM group (significant AUC changes observed 12 to 52 weeks).

Conclusion: The DJBL exerts prompt and robust effects on body weight and metabolic markers in obese subjects with or without dysglycemia. Enhanced incretin secretion and improved islet function may underlie some of the metabolic benefits. The unique combination of metabolic benefits following DJBL may offer an attractive alternative to pharmacological and surgical approaches.



Clinical Trial Registration Number: NCT00985491
Supported by: GI Dynamics funded clinical study

984

The duodenal-jejunal bypass liner (DJBL) effect of regression from prediabetes

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Background and aims: The endoscopically delivered duodenal jejunal bypass liner (DJBL) has demonstrated clinically meaningful metabolic effects in obese individuals with type 2 diabetes (T2DM). Prediabetes is a high risk state for the development of T2DM. The aim of this study was to examine the DJBL effects on glycaemic control, β cell function and sensitivity to insulin in a group of severely obese subjects with prediabetes.

Materials and methods: DJBL was implanted for one year in 61 subjects with severe obesity ($\text{BMI} \geq 35 \text{ Kg/m}^2$). Among these subjects, 18 were diagnosed with prediabetes. The included subjects were mostly females (mean \pm SD) age 36.8 ± 6.6 years, $\text{BMI} 39.6 \pm 2.9 \text{ Kg/m}^2$ and 17/18 were on metformin. Subjects were followed monthly for weight, fasting glucose, insulin and Hb A_{1c}. Insulin sensitivity and β cell function were estimated with HOMA calculator (www.dtu.ox.ac.uk/Homacalculator/index.php). Statistical analyses (ANOVA for repeated measures) were performed with Statistical Package for Social Sciences 17.0. A p -value of < 0.05 was considered significant.

Results: At implantation 17 out of 18 subjects were on anti diabetic medication that was withdrawn in 9/17 subjects, 8 subjects remained under metformin treatment. After implantation Hb A_{1c} decreased from $6.37 \pm 0.82\%$ (baseline) to $5.9 \pm 0.56\%$ at 4 weeks and remained below 6%. At 52 weeks was

5.88 ± 0.52 ($p < 0.001$) and fasting glucose decreased from $104 \pm 18 \text{ mg/dL}$ to $95.9 \pm 14.8 \text{ mg/dL}$ (NS). We observed an important change in insulin secretion reflected as improvement of insulin resistance. HOMA-IR decreased progressively from 3.1 ± 1.0 to 1.7 ± 0.7 ($p < 0.001$) and β cell function improved as insulin sensitivity increased from $37.5 \pm 11.9\%$ to $75.3 \pm 31.1\%$ ($p < 0.001$). At the end of the study 50% of subjects achieved Hb A_{1c} $< 5.7\%$.

Conclusion: The DJBL improved glucose metabolism in this group of prediabetic subjects, achieving normal glucose levels in half of them. The decrease of insulin resistance and recovery of β cell function observed in these patients opens new possibilities in order to obtain a regression from prediabetes.

Clinical Trial Registration Number: NCT00985491

Supported by: GI Dynamics

985

Acceptance of a truly non-invasive glucose monitoring device for home use

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Background and aims: Adherence to self-monitoring of blood glucose (SMBG) holds a key role in achieving tight glycemic control in diabetic patients. The conventional blood glucose monitoring methods require performance of invasive measurements, which involve pain and a rather complex multi-step procedure. Thus, despite the clear advantages of conducting SMBG, invasive monitoring suffers from under-utilization. A Non-invasive (NI) glucose monitoring approach is expected to encourage frequent self-monitoring, mainly due to a pain-free alternative. To further motivate utilization, a NI device should be user friendly and simple-to-handle by a lay person in home use. GlucoTrack[®] is such a NI, CE Mark approved glucose monitoring device. The device suitability for home utilization was evaluated in regards to the impact of an unskilled user upon the device accuracy, ease and simplicity of use and general user satisfaction.

Materials and methods: Series of clinical trials were conducted by third party to assess usability, user satisfaction and possible user impact. All trials began with individual calibration and brief training by a proficient team. Then, usability and general impression from the device were assessed based on feedback received from 72 educated (high-school and higher) users (Home group). 30 participants of the Home group used the device at home for 5–7 days after calibration. 42 other subjects of the Home group conducted the measurements by themselves for three more days simulating home alike environment. Then, user impact on the measurements' accuracy was evaluated by comparing performances when measurements were conducted by a proficient team (Clinic group, consisting of 135 subjects) with measurements conducted by the subjects themselves (42 of Home group subjects).

Results: Clarke Error Grid analysis shows 95.7% and 96.2% of the points in the clinically accepted A+B zones in the Clinic and Home groups, respectively. Mean Absolute Relative Differences of 31.6% and 30.7% were observed in these groups, respectively. 86% of all subjects expressed willingness to use the device regularly. 78% were pleased with the device. GlucoTrack display appeared clear and understandable to 91% of Home users. The operation instructions were clear to 97% of high school graduated Home subjects and to 88% with higher education. 79% and 85% (high school and higher educated Home users, respectively) found measurement performance to be simple. 74% and 69% claimed the device is easy to use among high-school and higher-educated, respectively.

Conclusion: GlucoTrack performances show negligent dependence upon user skills, based on similar accuracy when used by proficient and new users. Using the device yields positive user feedback, high satisfaction of usability, ease of use and users' willingness to increase the frequency of SMBG. These advantages, along with its painless nature of measurements and long term cost-effectiveness of use, suggest GlucoTrack as a utilizable home-use device for enhanced SMBG.

Clinical Trial Registration Number: NCT00889668

986

Improvement of metabolic control in type 1 diabetic children using ELKa toolset: a randomised controlled trial

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Background and aims: In intensive insulin therapy, prandial insulin doses should be calculated before each meal with carbohydrate (CHO) and fat/protein (FP) exchange counting. Therefore food weighing and complicated calculations need to be performed. ELKa system is an advanced toolset which helps performing calculation of food exchanges. It consists of ELKa software including database of various meals and nutrients and ELKaPlus digital kitchen scale. Among similar devices it is extraordinary due to opportunity of transmission weights in a real-time to a computer via USB port. After choosing the name of a particular product from the list, the program gives precise information about the amount of CHO and FP exchanges in a serving. It simplifies every-day calculations and it increases the precision of results. A number of our patients use the system and it appears to be useful accessory, but no clinical trials were performed yet. The aim of this study was to investigate the benefit of using ELKa toolset in comparison with standard method of CHO and FP counting on metabolic control in type 1 diabetic children.

Materials and methods: A randomized 26-week clinical trial was conducted on 106 patients (64 girls, 42 boys) aged 11 ± 4.2 years (range 1.8–17.2 years), with mean BMI 19.6 ± 3.4 (range 14.5–33.2), diabetes duration 5 ± 3.1 years (range 1–14.3 years), and HbA1c $7.5 \pm 1\%$ (range 5.1%–10%). All patients were treated with insulin pumps. Patients were randomly assigned into two groups: the group A ($n=53$) using ELKa system for food exchange counting and the group B ($n=53$) using standard method of food calculation. There were no significant differences between groups in mean baseline HbA1c levels (group A: $7.6\% \pm 0.1$; group B: $7.4\% \pm 0.1$; $p=0.395$), BMI (A: 18.4; B: 19.7; $p=0.165$), duration of diabetes (A: 4.3 years; B: 4.6 years; $p=0.265$) and insulin/kg/24h (A: 0.8; B: 0.8; $p=0.099$). Control group was slightly older (6.9 years ± 0.5 vs. 5.3 years ± 0.5 ; $p=0.042$). At follow-up visits after 3 and 6 months, HbA1c, BMI, daily insulin dose and blood glucose values were assessed. The group A also was asked about the frequency of using the toolset.

Results: 94 patients completed 26-week follow-up. There were no significant difference between groups with regard to HbA1c (group A - $7.4\% \pm 0.2$, $n=43$ vs. group B - $7.6\% \pm 0.1$, $n=51$; $p=0.222$). Patients who declared using the toolset everyday (86–100% of meals) or for the most part of the week (51–85% of meals) had significantly lower HbA1c (6.9 ± 0.2 , $n=21$) in comparison with the control group (7.6 ± 0.1 , $n=51$; $p=0.0005$). Moreover subgroup of patients who used ELKa only for 50–85% meals also achieved better result in a glycated hemoglobin than control group (HbA1c $6.8\% \pm 0.2$, $n=14$; $p=0.0009$).

Conclusion: Our results confirm the beneficial effect of using ELKa system in day-to-day calculations of CHO and FP exchanges. Diabetic children who used the system for more than 50% meals achieved better metabolic control than children who performed food calculations without this tool.

987

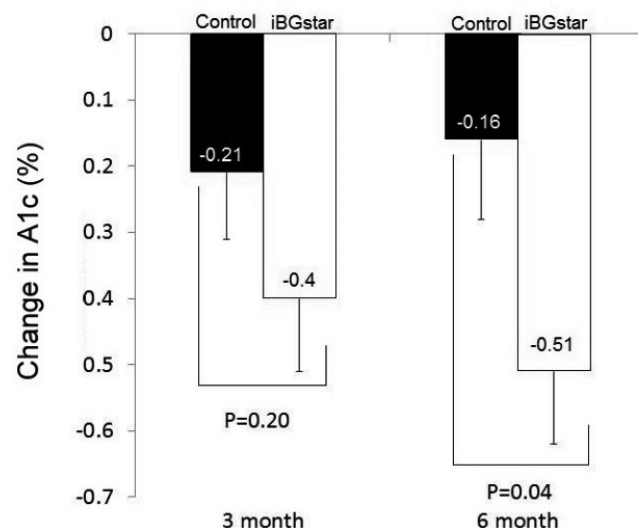
Role of mobile technology to improve diabetes care in adults with type 1 diabetes: the REMOTE-T1D studyS.K. Garg¹, W.R. Hiatt², P.A. Gottlieb¹, C.R. Beatson¹, F. Flacke³, V.N. Shah¹, J.K. Snell-Bergeon¹;¹Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, ²CPC Clinical Research, University of Colorado Denver, Aurora, USA, ³Sanofi, Paris, France.

Background and aims: This study evaluated the role of mobile technology to improve diabetes care in adults with type 1 diabetes (REMOTE-T1D). We hypothesized that the use of mobile technology (iPhone plus iBGStar®) will result in improvement in Patient Reported Outcomes (PRO).

Materials and methods: This single-center, prospective, 6-month, open-label, investigator-initiated pilot study enrolled 100 adult patients with type 1 diabetes. Patients were randomized in a 1:1 fashion to an intervention group using self-monitoring of blood glucose (SMBG) with iBGStar vs. SMBG with Accu-Chek Nano® (control). All subjects wore a blinded, continuous glucose monitor (CGM) for four separate 7-day periods. Primary outcomes included PRO (hypoglycemia fear questionnaire) and the secondary outcomes included glucose control parameters. Insulin doses, hypoglycemic episodes and self-monitored blood glucose (SMBG) measures were obtained at each visit. At the conclusion of the study, satisfaction with the device was ascertained.

Results: Baseline demographics and A1c values were similar between the two groups (mean SD \pm A1c 7.7 ± 1.0 and 8.0 ± 0.9 in the control and iBGStar groups, respectively, $p=0.15$). The Hypoglycemia Fear Scale improved compared to baseline in both groups to a similar extent at 3 months (-4.5 ± 9.5 in the iBGStar group vs. -3.0 ± 8.4 in the control group, $p=0.65$ between groups) and the changes remained similar between groups at 6 months (-3.9 ± 12.5 in the control group vs. -1.4 ± 10.0 in the iBGStar group, $p=0.31$). There was a significant improvement in change of A1c in the iBGStar group at 6 months when compared to baseline or the control group (Figure). The percentage of time spent in hypoglycemia (less than 70 mg/dL) as assessed by CGM readings was similar in the two groups throughout the study. There was a significant increase in insulin dose at 3 months in the iBGStar group (7.7 ± 20.9 units/day) but not in the control group (1.7 ± 18.3 units/day), with no difference in hypoglycemic episodes (21.5 ± 15.5 in the iBGStar group vs. 25.5 ± 31 in the control group, $p=0.48$) throughout the study. The number of SMBG tests per day was similar at baseline (4.8 ± 1.1 in the iBGStar group and 5.2 ± 1.1 in the control group, $p=0.07$) and decreased significantly from baseline in both groups at 6 months (-0.3 ± 0.09 in the iBGStar group vs. -0.4 ± 0.9 in the control group, $p=0.48$). At the exit interview, when asked whether they would prefer the iBGStar vs. their past SMBG device, 87.5% preferred the iBGStar.

Conclusion: We conclude that the use of iBGStar in this REMOTE-T1D study which allowed for easier communication of SMBG readings with the treating center by its email function, improved A1c at 6 months with no increased risk of hypoglycemia or change in PRO. This device was well-received by patients.

Change in A1c (%) in 3 and 6 Months

Clinical Trial Registration Number: NCT1825382

Supported by: Sanofi

988

Long-term tele-monitoring of patients with type 2 diabetes mellitus: results of the Greek pilot of the renewing health multicentre randomised control trialG.E. Dafoulas¹, A. Mavrodi², P. Gkiata³, H. Giannakakos⁴, P. Stafylas⁵, M. Delizona⁶, V. Aletras², P. Pechlivangolou⁷, K. Theodorou¹, G. Koukoulis⁶, A. Bargiota⁶;¹Faculty of Medicine, University of Thessaly, Larisa, ²Department of Business Administration, University of Macedonia, Thessaloniki, ³Intermunicipal Digital Community of Central Greece, Trikala, ⁴5th Regional Health Authority of Thessaly and Sterea, Larisa, Greece, ⁵Health Information Management SA, Brussels, Belgium, ⁶Department of Endocrinology and Metabolic Diseases, Regional University Hospital, Larisa, Greece, ⁷Toronto Health Economics and Technology Assessment Collaborative, University of Toronto, Canada.

Background and aims: Evidence is required to assess the impact of long term telemedicine use in treatment of patients with type 2 diabetes mellitus (DMT2). The aim of the present study was to examine the impact of a long-term telemonitoring program for patients with DMT2 on glycemic control,

health-related quality of life (HRQOL), physical activity and compliance with the mediterranean diet compared to usual care.

Materials and methods: In the Greek pilot of a prospective, randomized, single-blinded, multicenter, one year study 154 patients with DMT2 capable to use the telemonitoring device, with an HbA1c > 53 mmol/mol (7.0 % according to NGSP) were studied after they were randomly assigned in the telemonitoring group (IG), (N=74) and in the control group (CG), (N=80) and having signed the informed consent form. In the (IG) group patients' blood glucose profiles were collected weekly using a mobile phone health platform, for a period of one year. Allocated health professionals provided by phone the appropriate counseling on lifestyle and medication changes when required. Patients in (CG) group received usual care with face-to-face consultations. HRQOL was assessed using a generic (SF36v2) questionnaire and a disease-specific questionnaire, the Problem Areas in Diabetes (PAID) scale. Physical activity was assessed using the self-administered short form instrument. International Physical Activity Questionnaire (IPAQ) and the compliance with the Mediterranean diet using the Mediterranean Diet Quality Index (KIDMED) adapted for Greek adults.

Results: The table shows the outcome of the variables studied in both groups. A greater reduction in HbA1c was observed in the IG compared to the CG at the end of the study. There was a statistically significant improvement in the generic HRQOL in the MCS, in the disease specific HRQOL and the physical activity in the IG compared with the CG, but there was no improvement in KIDMED in neither or the two groups.

Conclusion: Our preliminary results indicate that in patients with DMT2, home telemonitoring is more effective than usual care in improving glycemic control with concurrent improvement in patients quality of life and increase of their physical activity. However home telemonitoring does not seem capable to empower patients with DMT2 with to follow a healthier diet.

Outcome	Intervention			Control		
	Baseline Mean (standard deviation) (SD)	After 12 month Mean (SD)	p-value	Baseline Mean (SD)	After 12 month Mean (SD)	p-value
HbA1c (%)	8.551 (1.38)	7.141 (0.61)	0.000	8.621 (1.43)	7.771 (0.78)	0.000
SF36-PSC scores	52.019 (4.34)	53.197 (2.97)	0.053	50.994 (6.12)	49.734 (5.08)	0.001
SF36-MSC scores	50.046 (8.42)	53.508 (6.54)	0.000	48.194 (10.17)	44.952 (8.90)	0.000
PAID scores (decrease denoted improvement)	17.698 (13.14)	10.793 (12.90)	0.000	22.013 (13.9)	26.363 (12.54)	0.000
IPAQ scores (MET-minutes/week)	7030.79 (5341.31)	7929.84 (5245.59)	0.008	6939.58 (6628.94)	4664.83 (4515.02)	0.000
KID-MED adapted for adults scores	0.24 (0.42)	0.16 (0.36)	0.096	0.16 (0.36)	0.06 (0.24)	0.008

Clinical Trial Registration Number: NCT01498367

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PS 081 Improvements in continuous glucose monitoring

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Accuracy of Continuous Glucose Monitoring (CGM) under free-living conditions during three home closed loop studies

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Background and aims: Closed loop systems modulate insulin delivery based on glucose levels measured by commercial CGM devices. We evaluated the accuracy of Freestyle Navigator II CGM (Abbott Diabetes Care, CA, USA) against the capillary glucose measured by the in-built glucometer of Freestyle Navigator II CGM using Freestyle Lite glucose strips. Data collected during three unsupervised randomised open-label crossover home closed-loop studies were pooled for the current analysis.

Materials and methods: Paired CGM and capillary glucose values (10,616 pairs) were collected from 57 participants with type 1 diabetes [41 adults (age 39±12 years, HbA1c 7.9±0.8%; mean±SD) recruited at five centres; 16 adolescents (15.6±3.6 years, HbA1c 8.1±0.8%) recruited at one centre]. Participants were instructed to calibrate the sensor according to manufacturer instructions. Numerical accuracy was assessed by relative absolute difference (RAD) (|sensor - capillary| / capillary) x 100% when capillary > 4.2 mM and by absolute difference (AD) |sensor - capillary| when capillary ≤ 4.2 mM. Clinical accuracy was assessed by the Clarke error grid analysis.

Results: Total duration of sensor use for whole cohort was 2,002 days (48,052 hours). The average number of capillary glucose values taken by adults and adolescents was 5.9 ± 2.0 and 4.3 ± 2.4 per day, respectively. Overall sensor accuracy for the entire capillary glucose range (1.1 to 27.8 mmol/L) was good with a median (IQR) RAD of 10.2% (4.5 - 19.0). Sensor accuracy stratified according to capillary glucose levels is shown in Table. Lowest RAD was observed in the hyperglycaemic range. Over 95% of pairs were in combined Clarke error grid zones A and B. Calculated per subject, median (IQR) RAD was 10.8% (8.7 - 11.5). Ninety percent of participants had a median ARD < 13.5%.

Conclusion: In a cohort comprising 57 subjects with T1D aged 12 years and older, we observed high and consistent accuracy of FreeStyle Navigator II. Particularly at glucose levels above 10 mmol/L, this facilitates safe operation of closed loop as otherwise insulin over-delivery due to overestimated glucose levels could lead to hypoglycaemia. Our results support the use of FreeStyle Navigator II in home closed loop studies and may contribute towards establishing performance criteria for CGM in home use.

Table: Numerical and clinical accuracy of FreeStyle Navigator CGM

	FreeStyle Navigator II
Number of paired points	10,616
Mean capillary glucose (SD)	8.6 (4.0)
ISO15197:2003 (%)	78.2
Clarke Error Grid (%)	
Zones A + B	78.6 +17.0 = 95.6
Zones C + D + E	0.4 +3.8 +0.2 = 4.4
Hypoglycaemia (≤ 4.2 mmol/L)	
Number of paired points (%)	1,279 (12%)
Median AD (IQR)	0.7 (0.3–1.3)
Mean AD \pm SD	1.0 \pm 1.2
Euglycaemia (4.3 to 10 mmol/L)	
Number of paired points (%)	5,945 (56%)
Median ARD (IQR)	10.4 (4.7–18.8)
Mean ARD \pm SD	14.4 \pm 15.5
Hyperglycaemia (>10 mmol/L)	
Number of paired points (%)	3,392(32%)
Median ARD (IQR)	8.0 (3.5–14.0)
Mean ARD \pm SD	10.5 \pm 10.3

All glucose levels are in mmol/L. AD=Absolute deviation (mmol/L), ARD=Absolute relative deviation (%)

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Comparison of paired and sensor-based glucose testing for meal bolus adjustment

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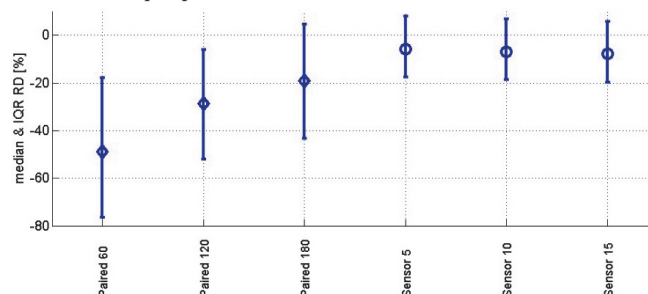
Background and aims: Meal boluses, whether based on carb counting or fixed bolus dosing, can benefit from periodic review of the post prandial glucose response. For carb counting, this can mean adjusting the carb ratio. For fixed bolus, this can be used to directly increase or decrease the fixed amount. The post prandial glucose response can be characterized by the difference between the peak glucose in response to the meal and the glucose at meal start. Two glucose testing methods for measuring this difference are considered. The first approach relies on a pair of discrete strip tests, one at the meal start and one at some fixed time after meal start. This method is confounded by the fact that peak meal times vary widely. The second approach utilizes sensor data which is sampled periodically. The benefit of this method is that the peak time can be estimated which provides a better estimate of the glucose difference. The precision of both methods will be compared, taking into account measurement error.

Materials and methods: Sensor data from a feasibility study of a 14-day sensor wear, comprising of 55 subjects with diabetes, is used. These data have a 1 minute sample rate and will represent “truth” for this analysis. True meal start and peaks have been previously identified. Paired testing is simulated using the truth data, with the first reading at the true meal start and the second reading at a predetermined fixed time interval (e.g., 60, 120 and 180 minutes). Sensor-based testing is simulated by decimating the truth data to longer sampling intervals (e.g., 5, 10, 15 minutes) and a meal detection algorithm is applied to these decimated data to estimate meal start and peak times.

Results: The figure summarizes the median and inter-quartile-range (IQR) of the relative difference (RD) between the “true” glucose meal response and the results from the six methods. The three sensor-based testing methods are shown to have much better precision than the paired testing methods; IQR RD for the best paired testing (Paired 120) is about 46% compared to about 25% for all of the sensor-based testing methods, and paired testing has substantially more negative bias. The difference in precision between the two test methods is primarily attributed to the wide variation in glucose meal

response from patient to patient; the sensor-based method estimates the peak time based on the data while the paired testing method assumes a fixed duration for the peak time. The figure also shows that performance for sensor-based testing is similar for sample periods up to 15 minutes.

Conclusion: The sensor-based testing method offers a more consistent characterization of meal response than the paired testing method, resulting in higher confidence that bolus adjustments can be made safely and effectively. The higher level of precision may allow dose titration that results in lower overall glucose levels with lower risk of hypoglycemia, but these results must be evaluated in prospective trials.



Supported by: Abbott Diabetes Care

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A cell-based hybrid biosensor for automatic real-time quality control of islets and sensing of insulin demand

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Background and aims: Glucose sensors have achieved considerable progress in the treatment of diabetes but require complex algorithms to match the complexity of everyday life. In contrast, pancreatic islets have been shaped during 0.5 billion years of evolution to secrete insulin not only in response to glucose, but also to other nutrients and to modulate their functioning to take physiological conditions into account via hormone receptors. Islets may provide the optimal sensor that uses changes in electrical activity as the first integrative signals of regulation. We have set out to develop recording and real-time analysis of these signals (i) for long-term analysis in drug testing, (ii) to provide a device for pre-transplantation control of donor islets and (iii) to advance in the development of a novel sensor for insulin demand.

Materials and methods: Mouse and human islets were cultured on multi-electrode arrays. Electrical activities received on each electrode were continuously amplified and analogically filtered. Output signals were recorded for off-line analysis or digitally converted and processed (for up to 60 electrodes in parallel) in real time using integrated circuits that were specifically designed on a custom electronics board.

Results: An acquisition system of islet electrical activity has been designed to process 60 channels in real time. This portable and autonomous system measures islet signals of extremely low amplitude (microvolts). Digital filters embedded on integrated circuits perform signal frequency separation, as well as frequency and statistical computation on individual signals. Extracellular recordings of human and mouse islet cells reveal two distinct glucose-dependent signals. According to pharmacology and experiments in Cx36-null mice, rapid action potentials (AP) are due to depolarization of single cells, whereas slower changes (slow waves, SW) result from coupling of multiple cells via gap junctions. In mouse islets, GLP-1 enhances SW frequencies at 8.2 mM glucose with maximal effect at 50 pM. In islets perfused with glucose, first increasing the concentration in small 0.5 mM steps from 3 to 11 mM and then decreasing by the same steps, both APs and SWs displayed a maximal effect at 10 mM. An exquisite glucose-dependency was present with a hysteresis shape, characterized by a shift of the EC₅₀ from 7.5 mM for the increasing phase of the glucose ramp to 8.5 mM glucose for the decreasing phase. Similar characteristics were observed in human islets. Moreover, our algorithms correctly blind ranked signals recorded at different glucose concentrations.

Conclusion: Glucose and GLP-1 signatures can be automatically computed from AP and SW signals. The islets provide natural integrated hysteresis

algorithms with rapid reaction to hyperglycemia and hormones as well as exquisite sensitivity to decreasing glucose levels, a safety latch against hypoglycemia. In the long-term, this system may also provide a unique biosensor for insulin demand.

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Hurdles in bioelectronics sensors: how to guide cells toward electrodes?

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Background and aims: We are exploiting the innate, multi-parametric sensing ability of islets in order to develop a hybrid bioelectronics glucose sensor. In our sensor, multielectrode arrays (MEA) record the electrophysiological signal produced by beta cells in response to multiple environmental factors (glucose, lipids, hormones, etc.). Signal quality, and consequently overall sensor performance, relies critically on close cell-electrode proximity. However, attempts to improve cell-electrode co-localization, via chemical/physical modification of electrodes or dielectrophoresis, lead to additional cell stress and convoluted setups. In contrast, we present here a non-invasive method of further exploiting the electrical properties of beta cells to guide them directly toward electrodes via simple electrophoresis.

Materials and methods: The electrophoretic motion of clonal beta cells (INS-1 832/13) (1E6 cells/mL) and murine islets (60 islets) in an electric field was investigated using a low conductivity, isotonic buffer (5 mM HEPES, 280 mM mannitol, 5 mM glucose). The electric charge at the surface of the INS-1 cells was measured in terms of their zeta potential using a Zetasizer Nano. Computational models describing the distribution of the electric field and predicting the path of cell motion were created to optimize electrode configuration. INS-1 cells were subjected to an electric field for 10 min and subsequently assessed via proliferation assay (MTT) after 24 h, glucose stimulated response test after 48 h, and analysis of electrophysiological activity after 48 h. Student's t-test was used in statistical analysis. Differences were considered significant ($p < 0.05$).

Results: Application of a minimum uniform electric field succeeded in displacing INS-1 cells and dissociated mouse islets (0.6 V/cm), as well as whole islets (10 V/cm), toward the positively charged electrode, suggesting that these cells possess a negative surface charge. This was confirmed by a zeta potential measurement of -27 ± 1.43 mV for INS-1 cells ($n=9$). Based on the results of the computational models, an electric field was generated in the MEA by applying an electric potential across neighboring electrodes in an alternating pattern. When 1.5 V was applied to the MEA, INS-1 cells and dissociated mouse islets mirrored the electrode pattern by repelling away from negatively charged electrodes and co-localizing directly over positively charged electrodes. Cells persisted in this pattern even after removal of the electric field. Subsequent assessment of viability, insulin secretion function, and electrophysiological activity revealed that cells were not functionally altered by the process as compared to controls ($n=3$) ($p > 0.1$).

Conclusion: Our findings demonstrate that INS-1 beta clonal cells and dissociated mouse islets can be driven directly to target electrodes by applying an electric field. The positive results of the viability tests, insulin secretion tests, and electrical recordings demonstrate that this technique is not detrimental to the health and function of beta cells. Furthermore, its application can be extended to other cell types and other bioelectronics sensors. We are currently investigating how to adapt this technique to improve the manipulation of whole islets, a task which is complicated by their higher density compared to single cells.

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Sensor and software use for improved glucose control in individuals with diabetes managed by multiple daily injections of insulin: the SIGN study

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Background and aims: Studies investigating the role of continuous glucose monitoring (CGM) to improve glycaemic control in type 1 and type 2 diabetes patients remain relatively scarce. Our study aims were to: 1) Assess whether subjects with type 1 (T1D) or type 2 diabetes (T2D) managed by multiple daily injection (MDI) of insulin could improve glycaemic control by understanding CGM data and reviewing ambulatory glucose profiles (AGP) with their clinician. 2) Identify device features and glucose profile characteristics that enable improved clinical outcomes.

Materials and methods: A UK, multicentre ($n=9$), 100 day study recruited 105 MDI treated diabetes patients aged 18–82 years with HbA1c of 58–108 mmol/mol. Subjects were randomised to control or intervention group; the control group used a standard self-monitoring blood glucose device, FreeStyle Freedom Lite. The intervention group employed a FreeStyle Navigator CGM and were asked to disable the glucose alarm function. At days 30 and 45, subjects reviewed AGP and summary statistics with a clinician and completed a questionnaire at the end of the study. Post-hoc exploratory analysis evaluated the frequency of high glucose variability, defined to be, have at least one period of the day (by each patient's typical meals and bedtimes) with the 50th – 10th percentiles > 4.72 mmol/L.

Results: In T1D intervention group ($n=29$), within group analysis showed significant reduction in hypoglycaemia (< 3.9 mmol/L) of 0.6 ± 1.4 hrs/day (mean \pm SD; $p=0.0472$) and a marginal decrease of 0.3 ± 1.0 hrs/day in time spent at < 3.1 mmol/L ($p=0.0760$), with no significant change in HbA1c (-3 ± 8 mmol/mol; $p=0.58$). In T2D intervention group ($n=28$), within group analysis showed a significant increase in time within 3.9–10.0 mmol/L of 1.4 ± 3.5 hrs/day ($p=0.0427$) associated with a reduction in HbA1c of 9 ± 12 mmol/mol ($p=0.0002$) without an increase in hypoglycaemia. Subjects agreed or strongly agreed with the statements: i) glucose profile was easy to understand [T1 ($n=29$) 93%, T2 ($n=29$) 93%], ii) need for management change was clear [T1 ($n=30$) 93%, T2 ($n=28$) 100%], iii) changes made after consultation with attending physician were helpful [T1 ($n=30$) 93%, T2 ($n=29$) 100%]. In the intervention group, incidence of high glucose variability was greater for T1 than T2 patients at baseline [T1 19/25 (76%), T2 13/28 (46%), $p=0.048$], with no significant changes detected at trial end [T1 19/25 (76%); $p=1.00$ and T2 10/28 (36%), $p=0.588$].

Conclusion: T1D and T2D subjects were able to understand glucose data independently and in review with their clinician. Using FreeStyle Navigator CGM over three months T1D subjects showed significantly reduced time in hypoglycaemia. T2D subjects showed significantly increased time in 3.9–10.0 mmol/L and reduction in HbA1c without increasing hypoglycaemia. Further study is required to understand how to best manage glucose variability to further improve glycaemic control.

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CGM is not a limiting factor in artificial pancreas systems

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Background and aims: CGM used for AP systems requires low glucose accuracy for safety, euglycemic and hyperglycemic accuracy to optimize insulin dosing determinations, and consistent performance across sensors and over time. A modified CGM designed specifically for the AP project was assessed in a clinical research study.

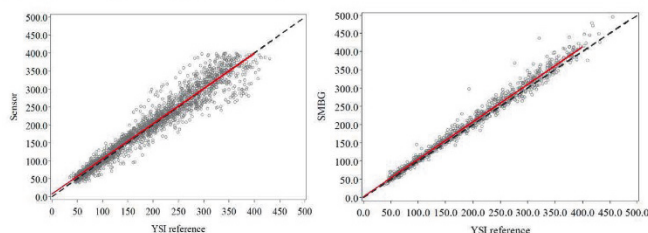
Materials and methods: The study enrolled 51 subjects from 3 US centers, 86% with T1D. Subjects wore sensors for up to 7 days and used self-monitored blood glucose to calibrate their CGM twice daily. Each subject was in-clinic for 12 hours on day 1, 4, or 7 to collect YSI reference venous glucose

every 15 minutes and capillary SMBG test every 30 minutes; glucose was manipulated to provide sufficient data in low and high glucose ranges.

Results: On average, the CGM provided glucose readings with a mean absolute relative difference (MARD) of 9% relative to temporally-matched reference YSI compared to 5% of that for the SMBG meter. The CGM readings were highly correlated with YSI with correlation coefficient of 0.97 comparing 0.99 of that for SMBG in the regression analyses. Using YSI reference, the CGM performed similarly as the SMBG meter. In particular, for hypoglycemia (< 70 mg/dL, i.e. 3.9 mmol/L), the mean absolute difference (MAD) was 6.4 mg/dL (0.36 mmol/L) for the CGM compared to 4.2 mg/dL i.e. 0.23 mmol/L for the SMBG; for hyperglycemia (> 180 mg/dL i.e. 10 mmol/L), the MARD was 8% for the CGM compared to 5% for the meter. The Clarke Error Grid (CEG) showed that 92% of CGM-YSI matched pairs fall in the clinically accurate A zone with 7% in the B zone, 0.0% in the C zone, and 0.5% in D zone for CGM; and 98.5% of SMBG-YSI matched pairs fall in the A zone with 1.1% in the B zone, 0.0% in the C zone, and 0.4% in the D zone.

Conclusion: The overall point accuracy, clinical accuracy, accuracy over the duration of wear, accuracy across the glycemic ranges, and reliability (98% of sensors lasted 7 days) were comparable to the SMBG meter. Accordingly, the CGM accuracy should not limit AP system development. Figure 1. Comparison of CGM-YSI vs. SMBG-YSI

Performance vs. YSI	CGM	SMBG
Temporal matched pairs (N)	2263	994
Pearson Correlation Coefficient	0.97	0.99
Mean Absolute Relative Difference (ARD)	9.0%	4.6%
MARD within Day 1/Day 4/Day 7	10.7%/8.0%/8.5%	5.3%/4.9%/6.6%
Mean Absolute Difference (MAD), at Hypoglycemia BG <= 70 mg/dl	6.4 mg/dL	4.2 mg/dL
MARD at Euglycemia 70 < BG <= 180	9.7%	6.1%
MARD at Hyperglycemia BG > 180 mg/dl	8.0%	4.8%
Overall CEG A+B Zones/A Zone	99.5%/92.4%	99.6%/98.5%
CG-EGA Zone Accurate Readings Hypoglycemia/Euglycemia/Hyperglycemia	95.6%/99.1%/99.2%	97.3%/99.7%/99.6%



Clinical Trial Registration Number: NCT02087995

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Glucose concentrations in blood and tissue: variable time lag

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Background and aims: Time constants dealing with carbohydrate metabolism are hot topics of diabetes physiology. Current methods derive glucose concentrations from different tissues using subcutaneous and whole blood measures. The aim of this pilot study was to assess the time delay between glucose concentration in blood and tissue in the course of increase and decrease.

Materials and methods: Three healthy volunteers, 3 persons with type 1 diabetes (DM 1) and 4 persons with type 2 diabetes (DM 2) underwent concurrent glucose measurements using standard equipment (1) continuous glucose monitoring system (CGMS), namely, sensor Enlite, transmitter Minilink and monitor Guardian, Medtronic Minimed, CA, USA, calibrated by glucometer Calla, Wellion, Austria, and (2) Beckman analyser to estimate venous plasma glucose concentrations. Samples were taken in fasting state (just before drinking 300 ml tea with 50 g glucose) and then 4 times (hourly). This test was performed on 3 consecutive days. Carelink Personal and MS Excel were applied to plot the 4-hour evolution of glucose concentration obtained by CGMS and by Beckman, respectively. In each volunteer the time difference between ascending and descending plots was estimated and averaged for healthy persons, for DM 1 and for DM 2, respectively.

Results: The results are summarized in the Table showing 1) the mean time delay of defined glucose concentrations between the methods, 2) the mean period of time from the start of consumption of glucose to the maximum difference between the rising glucose concentrations, 3) the mean period of time from the start of consumption of glucose to the maximum glucose concentration, 4) the mean delay between the decreasing glucose concentrations. The results are relevant to common practice.

Conclusion: In persons with diabetes glucose tolerance testing resulted in slower transport of glucose into subcutaneous tissue than in healthy subjects. The rate of change of glucose between blood and tissue varies, introducing errors in CGMS readings when calibrations are made in non-steady state conditions. CGMS can provide reliable plasma glucose concentrations but only if the system is calibrated during steady state. Hence, before each calibration an appropriate algorithm to confirm the presence of a steady state should be applied.

DG	Glucose tolerance test (GTT)			
	Delay between Beckman analyzer and CGMS - Medtronic - increase of concentration	Delay between start of GTT and maximum glucose concentration increase rate measured by CGMS Medtronic	Delay between start of GTT and maximum glucose concentration measured by CGMS Medtronic	Delay between Beckman analyzer and CGMS - Medtronic - decrease of concentration
	(min)	(min)	(min)	(min)
healthy	7	44	76	2
DM 1	19	55	99	-14
DM 2	26	53	98	9

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Glucose trend prediction in diabetic patients using Random Forests algorithm for analysis of CGM data

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Background and aims: Avoiding both hypoglycemia and hyperglycemia is the critical difficulty in the management of diabetes because of the impact of multiple factors on glycemic trends. Despite this, the development of new approaches to analysis of data obtained in continuous glucose monitoring (CGM) provides an opportunity for prediction of glucose level without quantifying of these factors. The aim of the study was to evaluate the possibility of using the Random Forest algorithm for glucose level prediction based on CGM data.

Materials and methods: Total 50 records of 48 h CGM obtained from patients with type 1 and type 2 diabetes mellitus were analyzed. Based on this dataset using Random Forests algorithm and R-STUDIO software a 50 linear regression models were created for each case individually. Programming the Random Forests module was performed using the author's modification of the algorithm of local search, which allows you to automatically find the optimal settings of the program for a particular purpose. Various predictive windows of 50-360 min were chosen to predict glucose trends in these diabetic patients. The algorithm was trained individually for each case on a set of randomly selected segments of the CGM record that total was 50% of the entire record. Subsequently, the algorithm was verified on the data not included in the training set. Model adequacy was evaluated by the difference between the predicted and actual value and also by the mean square error of all the predicted values at hypoglycemic and hyperglycemic ranges.

Results: Overall, a valid prediction has been provided at normal, hypoglycemic and hyperglycemic ranges in 50-180 min predictive window: the mean squared prediction error was 8.1, 8.6 and 9.8, respectively (p=0.0001).

Conclusion: Random Forests algorithm can be successfully utilized to predict glucose levels in diabetic patients based the CGM data. At the predictive window of 50-120 min (an important period related to postprandial glycaemia) the model has a good predictive accuracy. Models like these could be employed in a semiclosed-loop device that can provide better glycemic control in diabetic patients.

PS 082 Improving insulin pump treatment and continuous glucose monitoring use

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Time delay, reference measurement errors and number of reference measurements may strongly alter MARD values for CGM devices

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Background and aims: As a parameter describing the measurement quality of CGM systems most often the median (or mean) average relative deviation (MARD) is stated. For calculation of MARD paired CGM readings and results of reference blood glucose measurements are used. However, this comparison is affected by: a) measurements are made in different compartments (blood, interstitial fluid) which leads to a time-delay between the results; b) reference values are also affected by measurement errors; c) the selection and number of time points used for computation has an effect as well.

Materials and methods: In order to evaluate the effect of these factors we re-analyzed data from a clinical study in which the performance of 3 different CGM systems (two of each kind, 6 in total) was evaluated in 12 patients with type 1 diabetes. We systematically varied the time delay between the two measurement methods to find the delay which results in the minimal MARD. The result was verified using a correlation based time delay analysis. We simulated measurement errors of the reference method and formulated an optimization problem, which gives as a solution the reference measurement values inside the error bounds, which result in the minimal MARD values. We also systematically selected different time points of paired reference and CGM measurements which were excluded from the MARD computation: a) repeatedly excluding the time-point which contributes the most to the MARD; b) repeatedly excluding the time-point which contributes the least to the MARD. This results in two functional dependencies of the MARD from the nr. of samples used with the actual MARD value being in between the extreme cases.

Results: MARD values decrease from originally 16.5%, 12.6%, and 16.7% for the CGM systems by 1.5%, 1.7%, and 2.5% when compensating for the effect of the time-delay. MARD values varied in the worst case in the range of 11.0_21.8%, 6.9_16.3%, 10.7_21.6% when compensating for a 5% measurement error of the reference method and the estimated time-delay. The MARD values varied even more with different selections of the time-points of paired measurements: Reducing the number of paired measurements by 50% can result in an improvement (= lower value) by nearly 50% of the original MARD value. However, selecting different time points can also result in an MARD value twice as high as the original value.

Conclusion: MARD is greatly affected by various factors. Following a rigorous protocol that controls for certain factors that have an impact on MARD is crucial to enable uniform comparability among different clinical studies. POCT05-A guidelines should consider the effects discussed.

Supported by: Roche Diagnostics

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Using of RT-CGMS during perinatal period with special focus on newborn hypoglycaemia occurrence: our ongoing experience

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Background and aims: In babies of diabetic mothers perinatal complications including hypoglycaemia are still seen more frequently than in newborns of healthy mothers. In our study by using real time continuous Glucose monitoring system (RT-CGMS) we monitor pregnant women prior the labour and we continue to monitor their newborns after the birth as well to be able to follow complex glycaemia changes which influence the child prior and after

the birth and correlate them with the newborns' clinical status including hypoglycaemia occurrence.

Materials and methods: Newborns: We analysed by RT-CGMS 30 newborns of mothers suffering from pregestational diabetes, all mothers were treated by insulin. 23/30 of these children were born to type 1 diabetes (T1D) mothers, in 5/30 mothers were treated for T2D (type 2 diabetes) and in 2/30 cases the mother has MODY2 (maturity onset diabetes of the young). Median of gestation age in diabetes group was 37 weeks (range 31-38 weeks). Newborns were classified into four clinical status categories for further analysis. The first Enlite sensor was placed immediately after the birth (connected to Guardian real time device, Medtronic, Minneapolis, MN, USA). Eight term newborns born to healthy mothers were analysed as controls too. The median of monitoring in diabetes group was 5 days (range 3-8 days) and 4 days in controls (range 4-6 days). Mothers: in 9 T1D mothers their RT-CGMS records at least 3 days prior the labour and during the labour were available for the analysis too. In mothers the same type of RT-CGMS device was used as in newborns. Data were analysed by SPSS sw v.17. Notice - hypoglycaemia in newborns is considered as glycaemia ≤ 2.5 mmol/l.

Results: RT-CGMS revealed higher frequency of hypoglycaemia episodes in newborns of diabetic mothers (only 6/30 newborns of diabetic mothers were without any hypoglycaemia). These hypoglycaemia episodes were present not only shortly after the birth but after 3rd monitoring day as well ($p=0.01$). In control newborns hypoglycaemia episodes were present too (4/8) but during the first 4 days of life only and were less frequent and shorter. In T1D group the occurrence and severity of hypoglycaemia were influenced by: the presence of macrosomia ($p=0.022$), length of the newborn ($p=0.001$), maternal HbA1c in 3rd trimester ($p<0.001$), maternal weight gain ($p=0.02$), maternal total insulin dose ($p=0.001$) and by maternal age ($p=0.03$). Of particular importance to newborn's clinical status and hypoglycaemia occurrence seems to be the difference between maternal glycaemia one hour prior the child's birth and newborn's glycaemia measured immediately after the birth ($p<0.001$). Hypoglycaemia events were always confirmed in the laboratory and in general sensor readings correlated well with laboratory findings ($r=0.82$, $p=0.01$).

Conclusion: RT-CGMS can help not only to maintain maternal (near) normoglycaemia but is useful in revealing postnatal hypoglycaemia in her child which together with an adequate treatment can improve adaptation of newborns born to diabetic mothers.

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999

Efficacy and safety of insulin pump therapy in type 2 diabetes: the OpT2mise study

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Background and aims: Multiple daily injection (MDI) regimens may be offered to patients with type 2 diabetes (T2D) not responding to basal insulin therapy but offer only marginally improved rates of success. Alternative approaches to insulin therapy have included continuous subcutaneous insulin infusion (CSII) which have been studied in prior randomized controlled studies in T2D patients but produced conflicting results. This study is the first large multicenter, randomized, controlled trial aiming to compare the efficacy and safety of CSII vs MDI in insulin-using patients with T2D who failed to respond to a basal-bolus regimen after active insulin titration.

Materials and methods: Subjects with poor glycemic control ($n=495$) on multiple doses of insulin (MDI, basal-bolus using insulin analogs) were enrolled into a 9-week run-in period for insulin dose optimization (≥ 0.7 and ≤ 1.8 U/kg/d). Subjects showing persistent hyperglycemia ($HbA1c \geq 8\%$ and $\leq 12\%$) at the end of this period were then randomly assigned to switch to CSII or to continue with MDI regimens for 6 months. Both arms underwent double-blinded continuous glucose monitoring (CGM) assessments at baseline and 6 months. The primary endpoint was the between-group difference in mean change in HbA1c from baseline to 6 months.

Results: A total of 331 subjects were randomized (45.6% women, mean \pm SD age 56.0 ± 9.6 yr, BMI 33.4 ± 7.3 kg/m², diabetes duration 15.1 ± 8.0 yr, HbA1c $9.0 \pm 0.8\%$). Subjects assigned to CSII achieved significantly greater HbA1c reduction than MDI arm ($-1.1 \pm 1.2\%$ vs. $-0.4 \pm 1.1\%$, $p<0.001$), for a between-group difference favoring the CSII arm of -0.7% (95% CI, -0.9 to -0.4). The

percentage of subjects achieving HbA_{1c} <8.0% was 57% in CSII arm vs. 27% in MDI arm (OR=1.9, 95% CI 1.47 to 2.46, $P<0.001$). CGM analysis showed a significant reduction of duration of hyperglycemic events in the CSII arm compared to the MDI arm (-225 min vs -56 min, $p<0.001$) while no difference in the time spent in hypoglycemia was observed between both arms. At the end of the study, total daily insulin dose was 20.4% lower in the CSII arm than in the MDI arm (97 ± 56 U/d vs 122 ± 68 U/d, $p<0.001$), and there was no significant between-arms difference in body weight change (CSII, $+1.5\pm3.5$ kg vs MDI, $+1.1\pm3.6$ kg, $p=0.215$). No ketoacidosis occurred in either group, and one episode of severe hypoglycemia occurred in the MDI group.

Conclusion: The OpT2mise study demonstrates that CSII provides a significant advantage in glycemic control over MDI in T2D patients who fail to achieve HbA_{1c} target and therefore represents a new option for the treatment of T2D.

Clinical Trial Registration Number: NCT01182493

Supported by: Medtronic International Trading Sàrl

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Multi-centre closed-loop insulin delivery trial points to variability in keeping glycaemia in a pre-defined target range at the individual level

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Background and aims: Closed-loop insulin delivery aims at keeping blood glucose close to normal in patients with Type 1 diabetes (T1D) based on continuous glucose monitoring (CGM) and insulin administration according to a control algorithm. The performance of a model predictive control (MPC) algorithm in reaching a predefined glucose target range in a large number of patients at the individual level was investigated.

Materials and methods: Fifty-three T1D patients (27 adults: 15M/12F, age: 41 ± 11 , BMI: 24 ± 3 , HbA_{1c}: $7.7\pm0.6\%$, T1D duration: 25 ± 11 yrs; 26 adolescents: 13M/13F, age: 15 ± 1 , BMI: 23 ± 4 , HbA_{1c}: $8.1\pm0.9\%$, T1D duration: 8 ± 3 yrs) underwent a 22-hour (9AM–7AM) closed-loop insulin delivery trial in 7 Clinical Research Centers. Each admission included 3 meals with normal announced bolus before each meal and no exercise. The closed-loop system included the Dexcom Seven Plus CGM (San Diego, CA), the OmniPod insulin pump (Insulet Corp, Bedford, MA) and a laptop computer running an MPC algorithm. The inter-device communication was automated by the Artificial Pancreas Software. Control was evaluated for its ability to maintain glycemia in 71–180 mg/dl range from YSI-measured glucose every 15 min for 90 min post-meal and every 30 min between meals and overnight.

Results: For adults, mean glucose was 159 mg/dl and the mean percentage in target range was 66% overall, 59% daytime, and 82% overnight. At least one YSI value was >300 mg/dl and at least one ≤ 60 mg/dl in 22% and 19% of admissions, respectively. For adolescents, mean glucose was 166 mg/dl and the mean percentage in target range was 62% overall, 53% daytime, and 82% overnight. At least one YSI value was >300 mg/dl and at least one ≤ 60 mg/dl in 32% and 20% of admissions, respectively. In 33 cases, out-of-range excursions included only post-meal hyperglycemia. In 18 cases, hypoglycemia occurred as late post-meal events, following previous hyperglycemia in most cases. An alternative glycemic pattern, observed in 4 cases, included several hypoglycemic excursions during day and/or night without any post-meal hyperglycemia. Individual patterns of out-of-range excursions under closed-loop could be related to inaccurate estimations of insulin sensitivity and CHO/insulin ratio from pre-investigational individual data in open-loop control conditions.

Conclusion: Our data analysis points to the inter-individual variability of closed-loop glucose control contrasting with overall acceptable performance. It supports the necessity of both closed-loop studies in large populations including patients with various characteristics, and individualization of control algorithm parameters.

Clinical Trial Registration Number: NCT01271023

Supported by: JDRF

1001

The impact of exercise and reductions in insulin pump basal delivery implemented prior to exercise on circulating insulin levels in people with type 1 diabetes

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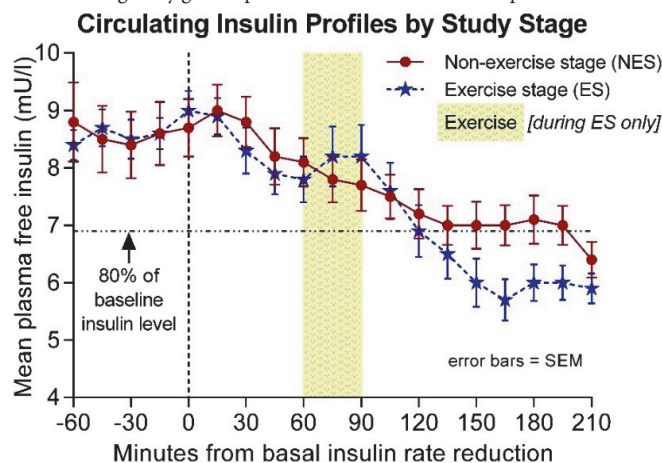
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Background and aims: Minimal data guides optimisation of insulin pump basal insulin infusion rate (BIIR) reductions prior to exercise. This study investigates the effect of a reduction in subcutaneous BIIR, and the effect of exercise itself, on circulating free insulin levels.

Materials and methods: In a two-stage randomised crossover study, 11 adults with type 1 diabetes using insulin pump therapy were studied fasting following a night on a single BIIR (6M/5F, mean \pm SD: age 43 ± 10 years, diabetes duration 22 ± 9 years, HbA_{1c} $7.1\pm0.5\%$, BIIR per kg body weight 0.011 ± 0.001 U/h/kg). Insulin, insulin pump and pump consumables were standardised. The exercise stage (ES) and non-exercise stage (NES) were in random order, separated by 1 to 6 weeks. In both stages, after 60 min of rest, BIIR was reduced by 50% for 210 min. Venous samples were collected at 15 min intervals from 60 min pre- until 210 min post-BIIR reduction. During NES participants were observed at rest without exercise. During ES participants exercised for 30 min on a stationary bicycle (target workload 65–70% of age-predicted maximal heart rate) commencing 60 min post-BIIR reduction, then rested for 120 min. Plasma insulin was measured by RIA; plasma was pre-treated with polyethylene glycol to precipitate bound insulin when antibodies were present. Data are presented as mean \pm SEM.

Results: Following the BIIR reduction in NES, free insulin levels reduced by $5.3\pm3.2\%$, $16.2\pm2.4\%$ and $16.4\pm3.5\%$ from baseline at 1h, 2h and 3h, respectively (baseline vs 1h $p=0.09$; 1h vs 2h $p<0.001$; 2h vs 3h $p=0.81$). The rate of insulin level decline post-BIIR reduction in NES did not correlate with baseline BIIR per kg body weight ($r=0.18$, $p=0.59$). Exercise for 30 min increased free insulin levels by 0.8 ± 0.3 mU/l compared to rest ($p=0.02$). Insulin levels subsequently declined more rapidly over the first hour post-exercise compared to NES (-2.2 ± 0.4 mU/l vs -0.7 ± 0.2 mU/l; $p=0.006$) [see figure].

Conclusion: The NES data demonstrate that when preparing for exercise, an interval of at least two hours is required after a 50% BIIR reduction to impact circulating free insulin levels. However, even by three hours post-reduction, insulin levels remain above 80% of baseline. Supplemental carbohydrate and/or a greater reduction in insulin dosing may be required to avoid exercise-induced hypoglycaemia. A transient increase in free insulin levels occurred during 30 min moderate-intensity exercise, consistent with initial enhanced absorption from the subcutaneous insulin depot due to increased blood flow during exercise. The subsequent more rapid decline in insulin levels over the first hour post-exercise could partly relate to this prior enhanced absorption. These findings may guide optimisation of BIIR reductions prior to exercise.



Clinical Trial Registration Number: ACTRN12613000581763

Supported by: ADS-Sanofi grant, Lynne Quayle Charitable Trust, sensors from Medtronic

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Factors associated with successful continuous subcutaneous insulin infusion therapy in type 2 diabetes patients - the OPT2MISE trial

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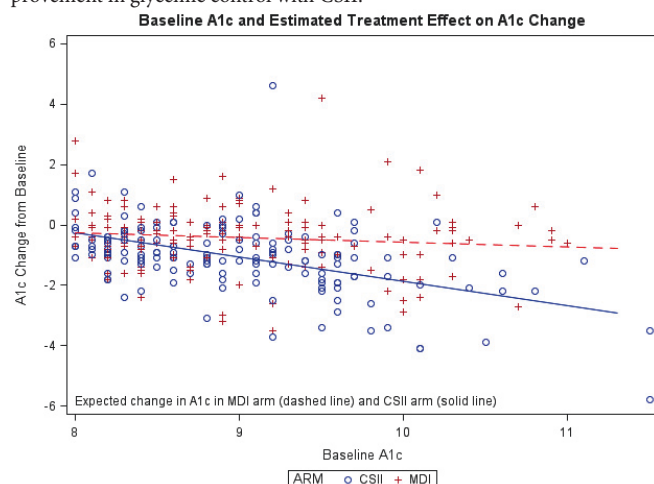
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Background and aims: The recent randomized study OpT2mise, demonstrated that insulin pump therapy (CSII) provided significant improvement in glycemic control, as compared with injection therapy (MDI) in type 2 diabetes (T2D). We aimed to assess the association between baseline or treatment related factors with glycemic improvements seen in the CSII arm of the study.

Materials and methods: Sub-optimally controlled (HbA1c $\geq 8\%$) T2D subjects, despite an MDI optimization period, were randomly assigned to CSII or continuing an MDI regimen. Simple and multiple linear regression analysis were performed to assess the association of A1c change at 6 months and factors such as age, gender, duration of diabetes, baseline A1c, BMI, treatment satisfaction score (DTSQs), total daily insulin dose, cognitive state score (MoCA) and glucose self-measurements (SMBG). Analyses were adjusted by baseline A1c.

Results: A total of 331 subjects were randomized (45.6% women, age 56.0 ± 9.6 yr, BMI 33.4 ± 7.3 kg/m², diabetes duration 15.1 ± 8.0 yr, HbA1c $9.0 \pm 0.8\%$). Subjects assigned to CSII achieved significantly greater A1c reduction ($p < 0.001$) and more treatment satisfaction ($p < 0.001$) than MDI arm. The effect of CSII and MDI in A1c change was dependent on baseline A1c ($p < 0.001$). CSII was superior to MDI in the observed range of baseline A1c values and the advantage of CSII over MDI increased with higher baseline A1c (between group difference = $-0.4 \pm 0.9\%$ for subjects with baseline A1c $\leq 9.0\%$ and $-1.0 \pm 1.4\%$ for subjects with baseline A1c $> 9.0\%$, $p < 0.01$ in both cases). Older age, longer duration of diabetes, low cognitive score and low SMBG use did not diminish the effect. In the CSII arm only 6 month satisfaction and number of boluses were associated with A1c reduction (a 3 point higher DTSQs was associated with a reduction of -0.02% in A1c and one additional bolus was associated with a reduction of -0.2% in A1c, $p < 0.01$).

Conclusion: CSII in sub-optimally controlled T2D patients was associated with greater improvement in A1c compared to MDI. Baseline A1c was a major predictor of glycemic response with improved CSII advantage over injection therapy for subjects with worst glycemic control. Further A1c improvement in CSII arm was associated with treatment satisfaction and number of boluses without increase in total daily dose. Although CSII treatment involves the use of advanced technology, patients who used lower frequency of SMBG, had lower cognitive state or older in age achieved comparable improvement in glycemic control with CSII.



Clinical Trial Registration Number: NCT01182493
Supported by: Medtronic Europe

1003

Effects of automatic insulin pump interruption on duration and weekly rate of nocturnal hypoglycaemic events in the ASPIRE In-Home Study

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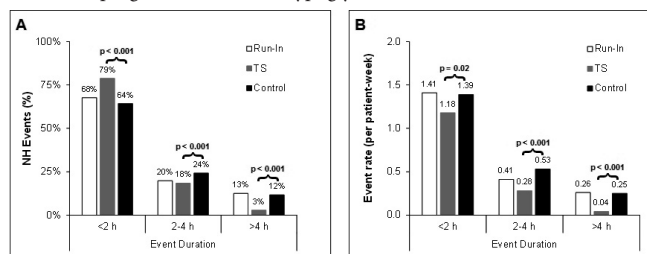
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Background and aims: The ASPIRE In-Home Study was designed to evaluate the Threshold Suspend (TS) feature of sensor-augmented pump therapy, which automatically suspends insulin delivery at a pre-set sensor glucose (SG) value. We evaluated the duration and weekly rate of nocturnal hypoglycemic (NH) events that occurred during the study.

Materials and methods: An NH event was defined as lasting >20 min with SG values ≤ 65 mg/dL that started between 10PM and 8AM. Subjects with type 1 diabetes with ≥ 2 NH events during a run-in phase were eligible. A total of 247 patients were randomized to SAP therapy with the TS feature (TS group, $n=121$) or without the TS feature (Control group, $n=126$). NH event durations throughout the 2-week run-in and 3-month study phases were enumerated and categorized as lasting <2 hours, 2–4 hours, or >4 hours.

Results: In panel A, 68% of NH events during the run-in phase lasted <2 h, 20% lasted 2–4 h, and 13% lasted >4 h. During the study phase in the TS group, 79% of the NH events lasted <2 h and 3% lasted >4 h, while in the Control group, 64% lasted <2 h and 12% lasted >4 h; these between-group differences were significant ($p < 0.001$ for each) and reflect shorter NH events in the TS group. In Panel B, the rate of NH events per patient-week was higher in the run-in phase than in the study phase, regardless of treatment group. During the study phase, the rate of NH events was significantly lower in the TS group than in the Control group, regardless of event duration. Compared to the Control group, the rate of NH events lasting <2 h was reduced by 15% ($p=0.02$), the rate of NH events lasting 2–4 h was reduced by 47% ($p < 0.001$), and the rate of NH events lasting >4 h was reduced by 84% in the TS group ($p < 0.001$). There was no severe hypoglycemia in the TS group and 4 events in the Control group.

Conclusion: In subjects with type 1 diabetes prone to hypoglycemia, use of TS was associated with favorable changes in the duration of NH events, with an increase in the proportion of short-duration events and a corresponding decrease in the proportion of long-duration events. Use of the TS feature was also associated with a reduction in the weekly rate of number of NH events, especially those lasting >4 h, suggesting a preventive effect and a reduction in the risk of progression to severe hypoglycemia.



Clinical Trial Registration Number: NCT01497938
Supported by: Medtronic Inc

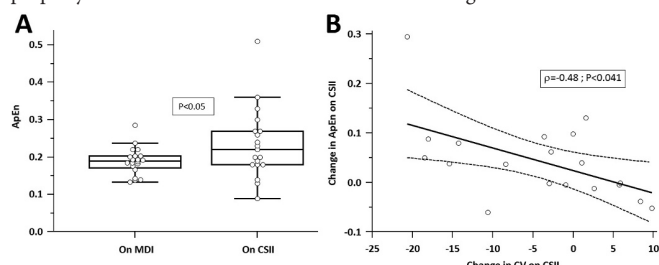
1004

Glucose variability outcome for type 1 diabetic patients switching to CSII: improved complexity patterns beyond glucose dispersion reduction
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Background and aims: Standard Deviation (SD) and MAGE are commonly reported indices of glucose variability (GV). However, they represent measures of glucose dispersion rather than true quantifications of instability. These indices are also directly proportional to the mean, making them inappropriate for comparisons of glucose profiles at different glucose levels. The aim of this work was therefore to study the evolution of GV in type 1 diabetic patients switching to continuous subcutaneous insulin infusion (CSII) in terms of both dispersion and complexity of the displayed patterns. For this purpose, we used metrics independent of the mean, as the Coefficient of Variation (CV), Approximate Entropy (ApEn) and the Detrended Fluctuation Analysis (DFA) method, which are, contrary to CV, sensitive to intrinsic complexity and self-similarities of glucose patterns rather than to value scattering.

Materials and methods: CV, ApEn ($m=1, r=0.2 \times \text{SD}$) and DFA- α were computed from blinded Continuous Glucose Monitorings (CGMs) of 48h duration (iPro2 with Enlite® sensors, Medtronic MiniMed, Northridge, CA) obtained from 20 adults with type 1 diabetes on MDI (baseline) and 6 months after initiation of CSII. Mean and SD of glucose, HbA1c, MAGE, the number of hypoglycemic episodes and time in hypoglycemia (TH) were also recorded. **Results:** CV was reduced by 4.9 ± 9.7 ($p=0.036$) on CSII, while change in CV (ΔCV) was strongly correlated with baseline CV (Pearson's $r=-0.77$, $p<0.0001$) but not with baseline HbA1c, glucose mean or SD. ApEn was found to increase on CSII (see panel A), indicating an increase in complexity of the glucose profiles. Although CV and ApEn were independent, changes in ApEn were inversely correlated with ΔCV (Spearman's $\rho=-0.48$, $p<0.041$), meaning that larger CV reductions were concurrent with greater increases of ApEn (see panel B). ApEn was found to be inversely correlated with DFA- α ($r=-0.93$, $p<0.00001$ on CSII) confirming that high ApEn glucose profiles obtain a DFA- α closer to 1 (DFA- α values ranged from 1.08 to 1.65 on CSII), meaning increased complexity with more self-similar structures and long-range correlations as described in CGM profiles of healthy subjects. Additionally, SD, MAGE, mean glucose, HbA1c and TH (<50 mg/dl) were reduced by 17 ± 24 mg/dl ($p=0.004$), 35 ± 52 mg/dl ($p=0.008$), 22 ± 40 mg/dl ($p=0.022$), $0.69 \pm 0.79\%$ ($p=0.001$) and 0.77 ± 1.21 h/day ($p=0.01$), respectively.

Conclusion: CV on MDI predicts GV outcome on CSII. Patients with the highest baseline CV demonstrated the greatest reduction on CSII. In these responders, CSII increased also Approximate Entropy. This brings the glucose control closer to physiology, not only in terms of dispersion, but also in terms of the complexity exhibited in the glucose patterns. To our knowledge, this property of the CSII treatment had never been investigated.



PS 083 On the way to optimise pump treatment

1005

PaQ®, a simple 3-day basal/bolus insulin delivery device, may optimise insulin delivery in type 2 diabetes as determined by continuous glucose monitoring

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Background and aims: PaQ (CeQur SA) is a simple patch-on device that provides set basal rates and bolus insulin on demand.

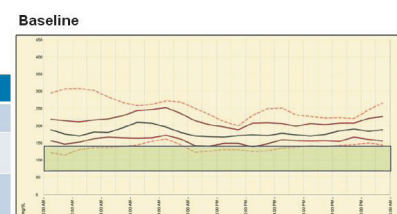
Materials and methods: A 6-week open-label single-arm study utilized blinded continuous glucose monitoring (CGM) to assess the effect of glycaemic control achieved with PaQ compared to multiple daily injections (MDI) of insulin. Twenty patients (age 59 ± 5 y, T2D duration 15 ± 7 y, A1C $7.7 \pm 0.7\%$), on a stable MDI regimen were enrolled and 18 completed. The study was comprised of three 2-week periods; baseline (MDI), transition to PaQ, and PaQ therapy. There was no attempt to treat to target. CGM data was analyzed by Ambulatory Glucose Profile (AGP) reporting system. AGP is used to graphically and statistically represent parameters of glycaemic control for each period of CGM and treat data collected over a period of days as if it were measured on the same (modal) day.

Results: An overall trend toward improved glycemia was seen with an average reduction in glucose exposure from 4024 to 3834 mg/dL/24hrs ($P=0.18$) and improved glucose fluctuations from 10.9 to 9.7 mg/dL/hr ($P=0.07$) while participants used the same total daily dose of insulin during Baseline and PaQ treatment periods. Analysis of by-patient CGM modal days revealed that 11 participants improved their glycaemic control (see accompanying figure).

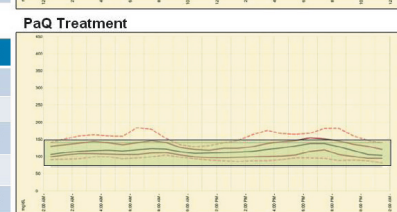
Conclusion: A larger study utilizing CGM is needed to further assess the insulin delivery potential of PaQ.

62 year old male
Diabetes for over 20 years
Baseline A1c - 8.1

Insulin	Baseline	PaQ
Total daily dose	45	48
Basal Insulin (units/day)	28	32
Meal time bolus/day	3.7	4.3



BG mg/mL	Baseline	PaQ
70 – 140 (%)	13	78
< 70 (%)	0	0.8
Mean	189	122
Stability	6.7	4.2
AUC (mg/dL * 24 hr)	4326	2896



This participant showed improvement in overall glycaemic control with all AGP parameters. The proportion of time in the target range (green box) increased from 13 to 78%. Overall glucose exposure decreased from 4326 mg/dL/24 hours to 2896 mg/dL/24 hours with no significant decrease in hypoglycemic events. This participant's AGP was close to those found in subjects with normal glucose tolerance.

Clinical Trial Registration Number: NCT01535612

Supported by: CeQur

1006

Association of late eating habit and sleep duration with glycaemic control in adult type 1 diabetes patients treated with insulin pumps

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Background and aims: The most important benefit of continuous subcutaneous insulin infusion (CSII) is customized, flexible basal and bolus dosing to meet patients' individual insulin requirements. This system of insulin delivery appears to offer not only improvement in metabolic control, but also increased physiological and psychological wellbeing. However, concerns have also been raised that, by enabling more freedom, CSII could potentially give rise "unhealthy" behaviors, which in turn could lead to the deterioration of

metabolic control. The goal of this research was to examine if sleep duration and late eating habit in CSII treated T1DM patients were associated with glycemic control as assessed by HbA1c level and mean self-monitoring blood glucose (SMBG) readings.

Materials and methods: We included 148 adult T1DM patients on insulin pump (100 females and 48 males) who filled a questionnaire concerning sleep duration (≤ 6 h and > 6 h) and late eating (snacking after 10 p.m. - rarely, sometimes, often). Other sources of information about the patients included available medical records together with the results of biochemical tests (HbA1c measured with high performance liquid chromatography method-HPLC), memory read-outs from insulin pumps, data from blood glucose meters. Data from personal insulin pumps and blood glucose meters were downloaded regularly to the computer and analysed via CareLink Professional (Medtronic) and Accu Chek 360 (Roche) software. Tests for differences between the two groups, univariate correlations analysis, and multivariate regression analysis were used.

Results: The patients' mean age was 26 yrs, mean T1DM duration 13.4 yrs, mean HbA1c level 7.2%, mean BMI 23.3 kg/m², while mean nighttime sleep duration was 7.2 h. Short sleep duration (26.3% of patients) as compared to long sleep duration was associated with worse glycemic control - HbA1c: 7.6 vs. 7.1%, $p=0.0293$; mean SMBG readings: 159 vs. 149 mg/dl, $p=0.0491$, respectively. Additionally, in a univariate correlation analysis we found an association between frequent late snacking (rarely: 54.1%, sometimes: 38.5%, often: 7.4%) and worse HbA1c level ($r=0.19$, $p=0.0287$) and SMBG readings ($r=0.21$, $p=0.0135$). In the multivariate regression analysis (dependent variables - age, T1DM duration, gender, BMI, sleep duration and frequency of late snacking), independent predictors for HbA1c were the patient's age ($\beta=-0.34$, $p=0.0013$), T1DM duration ($\beta=0.21$, $p=0.0440$) and sleep duration ($\beta=-0.23$, $p=0.0061$). The analyzed model was significant ($p=0.0017$). In the multivariate regression analysis for mean SMBG readings, the same variables except for T1DM duration were significant.

Conclusion: In summary, short sleep duration seems to be associated with poorer glycemic control in T1DM patients treated with CSII but late snacking is not.

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Factors predictive of overnight closed loop performance during free living in children and adults with type 1 diabetes

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Background and aims: Overnight closed-loop (CL) insulin delivery is feasible, safe, and effective in the home setting but outcomes vary between individuals. Understanding the factors influencing the performance of overnight CL under free-living conditions may help target those likely to benefit most while improving the control algorithm.

Materials and methods: We combined data collected during two randomized home studies in 16 adolescents (age 15.6 ± 3.6 years; mean \pm SD) and 24 adults (age 43.2 ± 11.7) with type 1 diabetes on insulin pump therapy (pooled HbA1c $8.0 \pm 0.8\%$; duration of diabetes 20.0 ± 14.0 years, BMI 24.3 ± 3.7 kg/m², total daily insulin dose 46.2 ± 17.3 U/day). Participants underwent, in random order, two periods of sensor augmented insulin pump therapy (SAP) or SAP combined with overnight CL insulin delivery, each lasting three-weeks (adolescents) or four-weeks (adults). Associations between baseline characteristics (age, HbA1c, duration of diabetes, BMI, total daily insulin dose and gender) and the performance of overnight CL (time in target, mean glucose) were examined using correlation analysis (Spearman's correlation coefficient, r).

Results: We analysed data on 866 CL nights at home. Lower baseline HbA1c ($r=-0.43$, $p<0.01$) was associated with lower mean glucose during CL, but not with greater time in the glucose target range (3.9 to 8.0 mmol/l). Longer duration of diabetes was inversely associated with time in target ($r=-0.44$, $p<0.01$). Other baseline characteristics such as gender, age, BMI, and total daily dose showed no association with either mean glucose or time in target. Baseline HbA1c (range 6.2–9.6%), age, duration of diabetes, and total daily dose were not associated with improvements (difference between conventional pump therapy and CL) in mean glucose or time in target during CL as compared to

SAP treatment. Females showed a greater improvement in mean overnight glucose during CL than males ($r=0.33$, $p=0.037$).

Conclusion: In adolescents and adults with type 1 diabetes, the extent of glucose improvements during CL is seen irrespective of baseline HbA1c. Reasons for reduced efficacy in patients with longer duration of diabetes as well as gender-specific differences in improved mean glucose warrant further investigations.

Clinical Trial Registration Number: NCT01440140, NCT01221467

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High reported treatment satisfaction in people with type 1 diabetes switching to latest generation insulin pump regardless of previous therapy

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Background and aims: Literature is lacking on how people with type 1 diabetes (T1D) cope with different insulin delivery therapies. CHOICE (Comparing perception of insulin therapies for T1D patients with the aim to Improve quality of Care) is an ongoing, multicentre, multinational, prospective observational study designed to fill this knowledge gap. A key aim is to compare treatment satisfaction (TS) between insulin pump naïve (IPN) and insulin pump experienced (IPE) people, after switching to a latest generation insulin pump (LGIP).

Materials and methods: People with T1D from 5 EU countries were included, comprising an IPN group and an IPE group. To optimise treatment homogeneity, only one LGIP (Animas Vibe) was used. An online participant survey including demographics, questions about LGIP use and the Insulin Treatment Satisfaction Questionnaire (ITSQ) was used to collect data. ITSQ is a psychometrically validated questionnaire available in different languages (including French and English), developed to evaluate TS with a range of insulin therapies, and provides a basis upon which new data are compared.

Results: Interim data (see table) are reported for participants ($n=190$; age range 12–79 years; disease duration 0–71 years) from 3 countries: France, the UK, and Ireland. There were no major cross-country differences between participants in gender, disease duration or previous treatment. French participants were 5 years younger than those in the UK and Ireland. Participants aged 12–29 reported higher fasting glucoses and higher pump use prior to LGIP. There was no significant difference in ITSQ overall satisfaction score with Animas Vibe, between participants previously treated with an insulin pump versus participants previously treated with multiple daily insulin injections. There was good internal consistency of the ITSQ Scale. The mean ITSQ-Sumscore (range 0–100) of 72.3 (SD 17.5) was similar to the 2004 confirmatory sample value (71.4; SD 16.5).

Conclusion: Data show that TS is very high in participants using an LGIP such as Animas Vibe regardless of previous insulin therapy. In this study, LGIP facilitated a good psychosocial functioning, important to optimal quality of life. Different people will use their insulin pump in different ways in the

context of their lived experience to support their own diabetes self-management. This study is ongoing, and final data are pending.

	Female % (n)	Male % (n)
Gender	61.6% (117)	38.4% (73)

	Mean	n	SD (+/-)
Age	39.7 years	190	15.8
Duration of disease	19.6 years	189	14.3
Number of glucose measurements per day	5.6	184	2.5
Number of correction boluses per week	9.4	181	8.8

	Treatment	% (n)
Previous insulin treatment	Pump	32.6% (62)
	Pen (pre-mixed)	4.7% (9)
	Pen (long-/fast-acting)	57.9% (110)
	Syringe/vial	3.2% (6)
	None	1.6% (3)

	Fasting BG	% (n)
Today's fasting blood glucose (BG) concentration by self monitoring BG	<7.0 mmol/L	49.7% (91)
	7.0-9.0 mmol/L	31.1% (57)
	>9.0 mmol/L	19.1 (35)

	Frequency	% (n)
Episodes of mild / moderate hypoglycemia since starting Animas® Vibe™ insulin pump	> than once a week	47.1 (88)
	Once a week	34.8% (65)
	Once a month	12.3% (23)
	< than once a month	4.8% (9)
	Never	1.1% (2)

Supported by: Animas Corp.

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OpT2mise study: the impact of insulin pump therapy on treatment satisfaction and resource utilisation in patients with type 2 diabetes

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Background and aims: Achieving glycemic outcomes in insulin-using type 2 diabetes (T2D) patients often requires multiple daily injections (MDI) with basal and bolus insulin types. Insulin pump therapy (CSII) has recently been evaluated in T2D patients in a large prospective randomized controlled trial. Patient outcomes have been found to be positively associated with treatment satisfaction and safety, contributing to improved health and reduced health-care costs. We assessed the effects of CSII on treatment satisfaction (TS) and on medical resource utilization, in suboptimally controlled T2D patients participating in the OpT2mise study.

Materials and methods: T2D subjects, suboptimally controlled (HbA1c ≥ 8%) despite an MDI optimization period, were randomly assigned to CSII or continuing MDI. The primary endpoint was the between-group difference in mean HbA1c change from baseline to 6 months. TS was measured by means of the Diabetes Treatment Satisfaction Questionnaire, status (DTSQs) and change (DTSQc). DTSQs questionnaires were collected at baseline and at 6 months; DTSQc was completed at 6 months. Data on medical resource use including diabetes-related hospitalizations, insulin usage and number of glucose testing strips were collected at each visit.

Results: 331 subjects were randomized (45.6% women, mean±SD age 56.0±9.6 yr, BMI 33.4±7.3 kg/m², diabetes duration 15.1±8.0 yr, HbA1c 9.0±0.8%). Subjects assigned to CSII achieved significantly greater HbA1c reduction than MDI arm (-1.1±1.2% vs. -0.4±1.1%, p<0.001). DTSQs scores at baseline and at 6 months were available for 283 patients. Mean DTSQs score increased significantly in the CSII arm after 6 months, with no change in the MDI arm (difference between groups = 5.0, 95 % CI=3.39-6.62, P < 0.001) with significant improvements in treatment convenience, flexibility, understanding of diabetes, willingness to recommend the treatment, satisfaction to continue treatment, and perceived frequency of hyperglycemia (all P < 0.001). There was no difference in the perceived frequency of hypoglycemia. Greater HbA1c decrease at 6 month was significantly associated with improved satisfaction, in the CSII arm only (P<0.05). Results were consistent in both DTSQs and DTSQc. Despite improved HbA1c outcome, mean total daily insulin dose (TDD) significantly declined from baseline to end of study in the CSII arm (112.1 ± 54 to 96.9 ± 55.8, P < 0.001). Mean TDD increased in the MDI arm over the same period (106.1 ± 49.2 to 121.7 ± 68.2, P< 0.05). Both groups had a statistically significant decline in daily strip use over the study. There was no significant difference in the number or length of stay of diabetes-related hospitalizations.

Conclusion: CSII therapy in suboptimally controlled T2D patients was associated with greater improvement in HbA1c at 6 months than patients continuing MDI therapy. In addition, CSII was associated with a significant increase in treatment satisfaction, with a significant reduction in total daily insulin usage. In T2D patients, suboptimally controlled with MDI therapy, CSII provides improved outcomes with greater treatment satisfaction.

Clinical Trial Registration Number: NCT01182493

Supported by: Medtronic International Trading Sàrl

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Insulin pump failures: a prospective study

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Background and aims: The rate of insulin pump failures in our centre had been evaluated by a prospective observational study published in 2009. During this six-year study (2001-2007), 36% of our pumps had broken down after a median time of 15 months. We had recorded substantial rates of severe pump failures with a complete failure in 44% of cases. The aim of this study was to update our data from 2008.

Materials and methods: This was a single-centre prospective observational study on 314 new pumps provided between 2008 and 2011 and maintained by the same institution. The pumps were used by adult type 1 diabetic patients treated in the Diabetes Department of a University Hospital in an ambulatory setting. There were 74 Animas®2020 and 240 Medtronic® including 40% Paradigm®Veo pump, 45% Paradigm®522/722 and 15% Paradigm®512-515/712-715. Pump failures were recorded until December 2013. We focussed on malfunctions directly related to the pump itself. Patients could not contact the manufacturer directly to exchange the pump. All pump failures were documented by a healthcare professional. We studied Kaplan-Meier survival curves. Logrank test, Cox model, t-test and χ^2 (or non-parametric tests when necessary) were used to compare the two brands. Statistical analyses were performed with the SPSS software (version 22; IBM, USA).

Results: The mean duration of pump use was 25±12 months (mean±SD) (CI 95%: 24-27) and did not differ between the two brands (p NS). Malfunctions occurred in 220 pumps (70%). Median time of survival was 27 months (CI 95%: 25-29). The rate of malfunction was 33 per 100 pump-years. We recorded 25 (11%) complete failures (pumps were immediately unusable), 16 (7%) alarms, 79 (36%) mechanical malfunctions that required pump replacement as recommended by the manufacturer, 94 (43%) minor defects. The cause of the defect could not be determined in 6 (3%) situations. Complete failures were mainly due to inoperative keypad (n=14); mechanical defects were mostly related to cracks in reservoir or battery compartment (n=60) and minor defects to screen display defects (n=48). Median time of survival in case of complete failure was 12 months (CI 95%: 9-16) versus 23 months (CI 95%: 21-25) in case of others defects (p<0.001). Survival curves of Medtronic® and Animas® pumps did not differ. Duration of survival of Paradigm®Veo pumps was longer than that of Animas® pumps (HR=0.65, CI 95%: 0.45-0.94, p=0.023). However the proportion of severe pump failures (complete failures

and alarms) was higher with Paradigm®Veo pumps (33%) compared to Animas® pumps (12%) ($p=0.007$).

Conclusion: Pump malfunctions remain common with modern pumps. In this study, we reported less complete failures than in our previous study. This may reflect an improvement of the pumps. However, the current high rates of mechanical and minor defects suggest changes in our practice and may be explained by the greater attention paid by patients and healthcare professionals to pump defects. A multi-centre evaluation of pump failures is needed.

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A randomised three-period trial to compare the pharmacodynamics and pharmacokinetics of different glucagon dosages at different blood glucose levels

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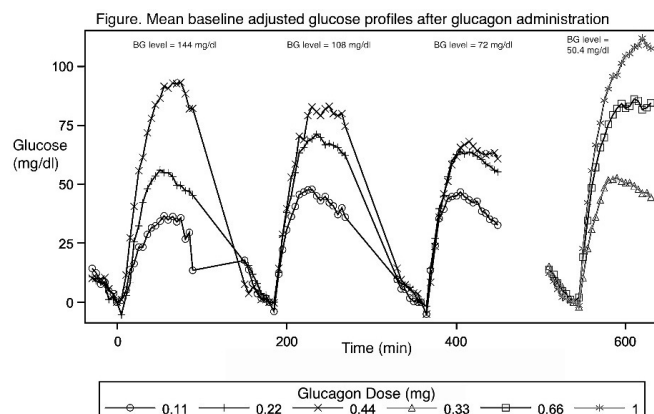
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Background and aims: Bihormonal closed-loop systems deploy the administration of glucagon in case of imminent or actual hypoglycaemia. The administration of glucagon in such a closed-loop system will not necessarily be limited to hypoglycaemia, but can also be done preemptively, trying to avoid hypoglycaemia. Thus more information is needed about the pharmacodynamic effects of small doses of glucagon at non-hypoglycaemic blood glucose (BG) levels. We evaluated the pharmacokinetics and pharmacodynamics of different dosages of s.c. glucagon at different BG levels.

Materials and methods: The study was conducted as an open, randomized 3-period cross-over experiment in six otherwise healthy patients with T1DM. At each of the 3 periods, different BG levels were established in 4 consecutive steps (144, 108, 72, and 50.4 mg/dl) and glucagon was given s.c. at each BG level in a randomized sequence (A: 0.11 mg at first 3 BG levels and 1.00 mg at the fourth, B: 3 x 0.22 mg and 0.66 mg, C: 3 x 0.44 mg and 0.33 mg).

Results: Glucagon raised BG in a dose-dependent fashion at all BG levels. Mean glucose excursion ranged from 48.7 mg/dl to 111.0 mg/dl (Figure). The response after repeated dosing tended to decrease only with the 0.44 mg glucagon dose (mean glucose excursion from 97.3 mg/dl to 71.0 mg/dl), possibly due to hepatic glycogen depletion. Time till maximum glucose concentration showed a dose dependency, with mean values increasing from 41.3 min to 79.2 min. Maximum plasma glucagon concentration increased with increasing glucagon dosage and was reached after around 10–25 minutes. For the 0.11 mg glucagon dose, plasma glucagon concentration tended to increase with decreasing baseline BG levels and in 3 patients no clear increase in glucagon concentration was seen at BG level of 144 mg/dl.

Conclusion: In conclusion, s.c. administered glucagon produces a predictable pharmacodynamic response at lower doses than the usual rescue dose and across a range of hypo- to hyperglycaemic BG levels. This supports the use of small glucagon doses in closed-loop systems to prevent impending hypoglycaemia. The results of this study can be used to further optimize the control algorithms of bihormonal closed-loop systems.



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The impact of insulin pump therapy on glycaemic profile of patients with type 2 diabetes: data from the Opt2mise study

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Background and aims: Previous studies did not conclusively demonstrated a benefit of continuous subcutaneous insulin infusion (CSII) in patients with Type 2 Diabetes (T2D) previously treated with basal-bolus multiple daily injections (MDI). The Opt2mise study is a multicenter, randomized trial comparing CSII to MDI in a large cohort of T2D subjects suboptimally controlled despite MDI therapy. Aim: To further understand the mechanism underlying the differences between both types of therapies, a study on the glucose metrics, as a secondary endpoint, was performed.

Materials and methods: Subjects with poor glycemic control ($n=495$) on MDI were enrolled into a run-in period for insulin dose optimization (Total Daily Dose ≥ 0.7 and ≤ 1.8 U/kg/d). Those showing an HbA_{1c} $\geq 8\%$ and $\leq 12\%$ were then randomly assigned to CSII or continuing on MDI for 6 months. Blinded Continuous Glucose Monitoring (CGM, iPro2, Medtronic) data was collected for a 6 day period before and 6 months after randomization and changes in glucose metrics were evaluated (Glucose exposure, Glucose Variability and Glucose Ranges).

Results: A total of 331 patients were randomized, aged 56.0 ± 9.6 , 45.6% women, BMI 33.4 ± 7.3 , diabetes duration 15.1 ± 8.0 yr, and HbA_{1c} $9.0 \pm 0.8\%$. CSII achieved significantly greater HbA_{1c} reduction than MDI ($-1.1 \pm 1.2\%$ vs. $-0.4 \pm 1.1\%$, $p < 0.001$). Data on CGM were available for 290 patients (143 and 147; 123 and 112 in the CSII and MDI arms at baseline and at 6 month, respectively). After 6 months, compared to MDI, 24h sensor glucose (SG) was reduced significantly more in CSII group (-17.1 mg/dl, $p < 0.05$), with less exposure to SG > 180 and SG > 250 mg/dl (-12.2% , $p < 0.001$ %, and -6.4% , $p < 0.05$), more time in target (70–180 mg/dl; 11.8 %, $p < 0.001$) and no difference in time exposure to SG < 70 mg/dl. Concerning glucose variability, there were no difference in 24h Standard Deviation (SD) SG, coefficient of variation (CV) or in mean amplitude of glucose excursions (MAGE).

Conclusion: In comparison with MDI, CSII treatment in suboptimally controlled patients with T2D provides a significant improvement in glucose profile with increased time spent in target, less exposure to hyperglycemia and without an increase in the risk of hypoglycemia.

Clinical Trial Registration Number: NCT01182493

Supported by: Medtronic Europe

PS 084 Supporting tools for insulin treatment

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Impact of a built-in insulin calculator feature on diabetes control: pilot study

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Background and aims: To evaluate the impact of the FreeStyle InuLinx meter with a built-in insulin bolus calculator on glycaemic control by observing time spent in euglycaemia (3.9 to 10.0 mmol/L) in the assessment phase vs. the baseline phase for the intervention group (primary endpoint), and measures of glycaemic control from a continuous glucose monitor (e.g. time spent in hypoglycaemia, secondary endpoints). Additional secondary endpoints considered in the study were: total daily dose of insulin (TDD), Hypoglycaemia Fear Survey (HFS), Diabetes Distress Scale (DDS) and Diabetes Treatment Satisfaction Questionnaire (DTSQ) results.

Materials and methods: The pilot study was a multicentre, randomised, two arm (control and intervention), parallel study conducted at 4 European study centres, with Type 1 diabetes subjects on Multiple Daily Injections (MDI, 3 or more per day) using U100 insulin (n=49). All subjects wore a masked FreeStyle Navigator Continuous Glucose Monitor (CGM) for 14 days (baseline phase) where upon randomisation, 33 entered the intervention group (one subject was excluded from analysis as their Navigator had alarms switched on for both CGM phases) and 16 the control group. The intervention group proceeded to use the InuLinx meter with built-in insulin calculator in either Easy (n=7, fixed dose meal time insulin calculator) or Advanced mode (n=25, rapid acting Insulin calculator with carb counting) for 45 days, the control group used a FreeStyle Freedom Lite meter for 45 days. After this time both arms continued with their designated meter together with a masked CGM for 14 days (assessment phase).

Results: When comparing baseline versus assessment phase in the intervention group, no significant difference in time spent in euglycaemia (3.9 to 10.0 mmol/L) was found (primary endpoint). Study statistics found a decrease in the number of excursions per day <3.9 mmol/L (-0.24, p<0.05) and on the DTSQ, the treatment satisfaction scale was significantly improved by 2.0 points within the intervention group. When separated into InuLinx easy mode vs. advanced mode, there was a significant reduction of the number of excursions per day <3.9 mmol/L (-0.5, p<0.05) compared to baseline when the meter was used in easy mode. When comparing the intervention group versus the control group, the use of the InuLinx meter in easy mode resulted in a significant reduction of time spent <3.9 mmol/L (-1.1 hours/day from baseline of 2.2, p<0.05). The HFS found that using the InuLinx meter in advanced mode, resulted in a significant reduction in the total worry score (by 3.9, p<0.05) compared to the control group. TDD of insulin increased significantly (p<0.05) for subjects in the intervention group compared to the control group.

Conclusion: The indications from this pilot study are that use of a meter with a built-in insulin calculator can lead to improvements in diabetes control, particularly in reducing the prevalence of hypoglycaemic events, can reduce worry of hypoglycaemia and improve treatment satisfaction. It is postulated that patients first try to avoid hypoglycaemia and next focus on reducing hyperglycaemia; hence the relatively short length of the study may have prevented an increase in time in euglycaemia.

Clinical Trial Registration Number: ADC-PMR-INX-11012

Supported by: Abbott Diabetes Care

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Does frequent use of an automated bolus advisor improve glycaemic control in pediatric patients treated with insulin pump therapy?

First results of the BABE study

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Background and aims: Studies have shown that use of an automated bolus advisor improves glycemic control in type 1 and insulin-treated type 2 diabetes; however, the relationship between frequency of bolus advisor use and glycemic improvement has not been well studied.

Materials and methods: The Bolus Advisor Benefit Evaluation (BABE) study was a single-center, retrospective cohort study that assessed the impact of frequent use of the Accu-Chek Aviva Combo system bolus advisor (BA) feature on glycemic control among pediatric type 1 diabetes patients on insulin pump treated at a pediatric diabetology clinic in Germany. Measurements of HbA1c, hypoglycemia (<60 mg/dL), therapy changes, mean blood glucose and glycemic variability (SD) were assessed at baseline and after 3 and 6 months of BA use. A total of 104 pediatric patients with mean (SD) baseline: HbA1c 8.0(1.6)%, age 12.7(4.9) years, diabetes duration 46.7(43.7) months, and 58.7% female were assessed. Baseline differences in HbA1c, diabetes duration and age were accounted for by ANCOVA analyses.

Results: After 6 months of BA use, 71 patients reported high frequency (HF) of device use (≥50%) versus 33 patients who reported low frequency (LF) use (<50%) during the study period. Mean (SE) HbA1c among HF users was significantly lower than LF users: 7.5(0.1)% vs. 8.0(0.2)% (p=0.0252). There was with no between-group difference in the percentage of hypoglycemia values (<60 mg/dL) within 30 days prior to 6-month visit: 5.5 (4.5)% vs. 5.9(5.8)%, p=0.6526. More HF than LF users received therapy changes during the 6-month period: 62(87.3%) vs. 22(66.7%), p=0.0174. HF users showed significantly lower mean blood glucose (164.4 [29.78] vs. 194.5 [38.70] mg/dL, p<0.0001) and less glycemic variability as assessed by standard deviation (80.1 vs. 100.6, p=0.0001) than LF users.

Conclusion: Frequent use of an automated bolus advisor was associated with significant improvements in glycemic control and more therapy changes with no increase in hypoglycemia associated with BA use in pediatric patients treated with CSII therapy.

Supported by: Roche Diagnostics

1015

A new assessment tool to measure the ability of bolus calculation and carbohydrate estimation (SMART) in people with diabetes performing an intensive insulin therapy

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Background and aims: Intensive insulin therapy relies on correct prandial insulin dose adaptation dependent from current glucose level, amount of planned carbohydrate intake and the consideration of other situational factors like physical activity or circadian fluctuation of insulin sensitivity. People with diabetes and intensive insulin therapy should be able to estimate carbohydrates and calculate insulin bolus correctly, while regarding the factors mentioned above. An assessment tool for the measurement of the ability of carbohydrate estimation and bolus calculation is missing. The objective of this study was the development and psychometric evaluation of an assessment tool for carbohydrate estimation and bolus calculation ("aSSessment of the Ability of Bolus Calculation and Carbohydrate estimation" SMART). Of special interest were the associations of both abilities with glycaemic control.

Materials and methods: The SMART consisted of one scale for the assessment of bolus calculation (BOLUS) with 10 items and a scale for carbohydrate estimation (CARB) with 12 items. People with type 1 or type 2 diabetes on an intensive insulin regimen were invited to participate. HbA1c and stored data of blood glucose meters were used to determine glycaemic control.

Results: 411 patients participated (age 42.9 ±15.7, 58% female, HbA1c 8.6 ±1.8%, 28% with CSII-treatment) and approx. 56,000 blood glucose meter readings could be obtained. The reliability of both scales was sufficient (Cron-

bachs alpha for BOLUS $r = 0.78$ and the CARB $r = 0.67$). Better bolus calculation was associated with a higher level of education ($r = 0.24$, $p < .05$), lower HbA1c ($r = -0.27$, $p < .05$), lower mean blood glucose ($r = -0.29$, $p < .05$), and a lower standard deviation of blood glucose values ($r = -0.43$, $p < .05$). Better carbohydrate estimation was associated with a lower body mass index ($r = -0.2$, $p < .05$), lower mean blood glucose ($r = -0.3$, $p < .05$), a lower frequency of hyperglycaemia ($r = -0.27$, $p < .05$), and a higher frequency of euglycaemia ($r = 0.26$, $p < .05$). Patients with an insulin pump were better on both scales than patients with multiple daily insulin injections (BOLUS: 7.2 ± 2.4 vs. 6.4 ± 2.7 , $p < .01$; CARB: 7.8 ± 2.1 vs. 7.1 ± 2.6 , $p < .01$). Patients with previous diabetes education performed significantly better on both scales (BOLUS: 6.8 ± 2.5 vs. 5.7 ± 2.8 , $p < .01$; CARB: 7.4 ± 2.4 vs. 6.5 ± 2.6 , $p < .01$).

Conclusion: SMART provides a reliable and valid assessment of the ability to estimate the correct amount of carbohydrates and to calculate the appropriate prandial insulin dose. SMART is also sensitive to depict effects of diabetes education and of CSII treatment in comparison to multiple daily insulin injections. In summary SMART can assist the identification of people with diabetes on an intensive insulin regimen, who are in need for improvements in carbohydrate estimation and/or calculation of prandial insulin dose.

Supported by: Roche Diagnostics

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A clinical evaluation of blood sampling practices in patients with diabetes: impact on fingerstick blood volume and pain

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Background and aims: There is a perception that patients with diabetes struggle to produce sufficient blood to fill glucose test strips, including strips with 1 µl fill requirements. The purpose of this study was to determine the volume of blood expressed when these patients perform routine fingersticks using their own lancing device and sampling technique, and to evaluate the relationship between blood volume and pain. In addition, a survey was used to explore patient perceptions and preferences regarding blood sampling.

Materials and methods: Each of the 64 subjects (type 1 or type 2 diabetes mellitus) performed 8 fingersticks using their own lancing device, preferred depth setting and lancing technique. Eight different commercially available lancing systems were used (8 subjects/system). Blood volume and perceived pain were recorded after each fingerstick. Subjects used the same depth setting typically used at home to lance. In addition, they were instructed to express capillary blood from their fingertips using the same technique they typically use at home and consistent with their specific lancing device instructions.

Results: Average blood volume generated across all subjects was 3.1 µl (SD=2.1 µl, from 512 fingersticks) with 97% of subjects expressing an average of ≥ 1.0 µl of blood. There was no significant contribution due to lancing system on individual or mean blood volume ($F=1.623$; $p=0.15$). There was no correlation between pain response and the volume of blood expressed ($r=-0.054$; $p=0.67$). Similarly, there was no statistically significant correlation ($r=0.164$; $p=0.19$) between lancing depth setting and the pain reported by the subject. Nearly all subjects agreed they could easily and comfortably obtain a 1 µl blood sample, and most subjects actually preferred a larger drop size when doing blood glucose tests to ease sampling and avoid wasting strips.

Conclusion: These results provide evidence across 8 lancing systems that challenges the current perceptions that patients with diabetes struggle to produce sufficient blood samples to fill most test strips, including those with 1 µl fill requirements, and that obtaining larger volumes of blood is more painful.

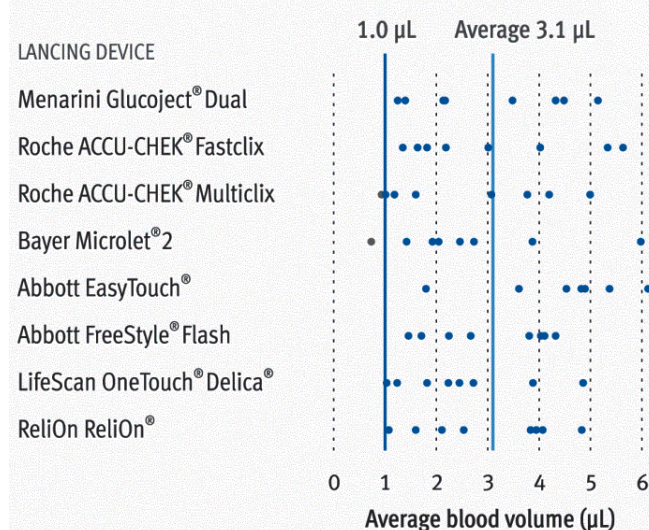
These results are consistent with previous literature suggesting patients (1) derive no real benefits from test strips with very low blood sample volumes and (2) generally prefer a blood drop size that enables them to confidently fill their test strip.

Average fingerstick blood volume

97% subjects expressed > 1 µl blood

Data shown for all 64 subjects, organized by lancing device

Each point represents the average of 8 fingersticks per subject



Clinical Trial Registration Number: NCT01914302

1017

System accuracy evaluation of 10 SMBG systems with 3 lots each following ISO 15197:2013 against 2 different comparison methods

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Background and aims: High quality blood glucose (BG) monitoring systems are required for adequate self-monitoring of blood glucose (SMBG). The international standard ISO 15197, which was revised in 2013, stipulates performance requirements for SMBG systems. Prior to the revision, ISO 15197 prescribed a system accuracy evaluation against the manufacturer's measurement method. This was changed so that any method traceable according to ISO 17511 may be used. In order to investigate possible effects on apparent SMBG system accuracy, 10 SMBG systems with 3 lots each were evaluated against 2 comparison methods.

Materials and methods: In this study, system accuracy of 10 SMBG systems was evaluated with 3 different reagent system lots each. Each lot was tested on 100 capillary blood samples from different subjects following investigational procedures of the international standard ISO 15197:2013. Comparison measurements were performed with the YSI 2300 STAT Plus™ glucose analyser (glucose oxidase method (GOD)) and the Cobas® c111 (hexokinase method (HK)). Both methods were traceable according to ISO 17511 as required by ISO 15197:2013. Compliance with accuracy criteria stipulated in ISO 15197:2013 was assessed for each reagent system lot by calculating the percentage of results within $\pm 15\%$ or within ± 15 mg/dL of the comparison method results for BG concentrations above or below 100 mg/dL, respectively, and by calculating the percentage of results within Consensus Error Grid zones A and B across all 3 lots of a system. Additionally, accuracy criteria of ISO 15197:2003 were applied by calculating the percentage of results within $\pm 20\%$ or within 15 mg/dL of comparison method results above or below 75 mg/dL, respectively.

Results: Seven of 10 systems (Accu-Chek® Performa; BGStar®; Contour® next USB; Contour® XT; FreeStyle InsulinX; mylife™ Pura™; MyStar Extra®) fulfilled the minimum accuracy criteria of ISO 15197:2013 (at least 95% of results within $\pm 15\%$ or ± 15 mg/dL of the comparison method results and at least 99% within Consensus Error Grid zones A and B) with all 3 tested reagent system lots, independent from the comparison method applied. In

the evaluation against the respective system's manufacturer's measurement method (GOD for 7 systems, HK for 3 systems), individual reagent system lots showed 90% to 100% of results within $\pm 15\%$ or ± 15 mg/dL of the comparison method results, with 23 of 30 lots having at least 95% within these limits. When evaluated against the alternate comparison method, between 83% and 100% of results were within these limits for individual lots and 25 of 30 lots showed at least 95% within the limits. Eight of 10 systems fulfilled the minimum accuracy criteria of ISO 15197:2003 (at least 95% of results within $\pm 20\%$ or ± 15 mg/dL of the comparison method results) against the respective manufacturer's measurement method. Individual lots showed between 94.5% and 100% of the results within these limits and 28 of 30 lots showed at least 95% within these limits.

Conclusion: In this evaluation, 7 of 10 systems met the minimum system accuracy criteria of ISO 15197:2013. Analysis of individual reagent system lots showed that specific evaluation results differed depending on the comparison method. These data suggest that the comparison method used in an evaluation can have an influence on an SMBG system's apparent system accuracy. Investigator-initiated study supported by Sanofi

1018

Is fingerstick calibration beneficial for stable and consistent sensors?

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Background and aims: Subcutaneously inserted glucose monitoring systems typically require fingerstick calibrations. Calibrations allow for sensor specific adjustment and can correct for any sensor drift over the wear duration. When sensors can be manufactured with a sufficiently low in-vitro lot sensitivity CV and can remain stable in-vivo, fingerstick calibration may no longer be beneficial. Universal calibration provides a shared, one-time calibration factor for all sensors from the same lot. This analysis computes the expected average and expected 90th percentile sensor performance in fingerstick and universal calibration studies. Per-sensor mean absolute relative difference (MARD) distribution is used for the comparison.

Materials and methods: A total of 33 subjects with diabetes were enrolled to wear four sensors simultaneously. Sensors from a lot with low in-vitro sensitivity CV ($\approx 2.9\%$) were used in the study. To examine the effect of fingerstick error, an emulator that allows raw sensor data from the actual clinical study to be recalibrated with any meter was used. No difference in per-sensor MARD distribution was observed when the same meter type from a different clinical study data was used for calibration (z-test p-value = 0.80). The fingerstick calibration study was repeated 1000 times using a simulated 15/15 error meter (i.e. 95% of the values within 15 mg/dL below 100 mg/dL reference value, and within 15% at or above 100 mg/dL). The universal calibration study is also repeated 1000 times, each time randomly selecting 3 sensors and using the median of the per-sensor sensitivities to calibrate the rest.

Results: Each study generated a distribution of per-sensor MARD values. After repeating the study 1000 times, the range of average per-sensor MARD from the fingerstick calibration studies has a mean and standard deviation of 13.7% and 0.24%, respectively (Table 1). The table also shows the average from the 1000 universal calibration studies, as well as the 90th percentile of both studies. The per-sensor MARD distribution of the universal calibration studies, both in terms of the average and 90th percentile, is statistically better than that of the fingerstick calibration studies ($p < 0.0001$).

Conclusion: Fingerstick calibration can be a necessary element to ensure good and consistent sensor performance. However, stable sensors with low in-vitro sensitivity variation do not appear to benefit from fingerstick calibration.

	per-sensor MARD (from 1000 studies)	
	average [mean \pm standard deviation]	90 th percentile [mean \pm standard deviation]
Fingerstick calibration (simulated 15/15 error meter)	13.7% \pm 0.24%	18.9% \pm 0.63%
Universal calibration (randomized median of 3 sensors)	13.5% \pm 0.88%	18.0% \pm 1.61%
z-test p-value	<0.0001	<0.0001

Supported by: Abbott Diabetes Care

1019

Standardised procedure for the assessment of new-to-market continuous glucose monitoring (CGM) systems (Space2)

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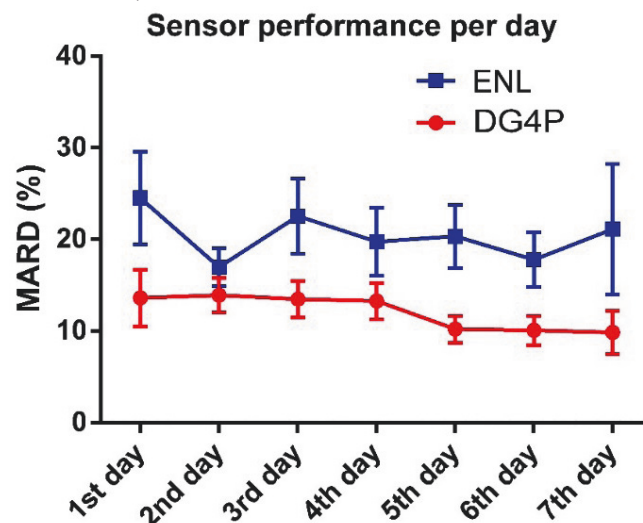
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Background and aims: This study assessed the accuracy and reliability of the two most widely used continuous glucose monitoring (CGM) systems.

Materials and methods: We studied the G4 Platinum (DG4P) and Veo Enlite (ENL) CGMs, in 24 patients with type 1 diabetes. CGMs were tested during six day home use and a nested six hour clinical research center (CRC) visit. During the CRC visit, frequent venous blood glucose samples were used as reference while patients received a meal with an increased insulin bolus to induce an aggravated postprandial glucose nadir. At home, patients performed at least six reference capillary blood measurements per day. Wilcoxon signed-rank test was performed using all data points ≥ 15 min apart.

Results: Overall Mean Absolute Relative Difference (MARD) (SD) measured at the CRC was 13.6% (11.0) for DG4P and 16.6% (13.5) for ENL ($p < 0.0002$, CI $\Delta = 1.7$ -4.3%, $n = 530$). Overall MARD assessed at home was 12.2% (12.0) for DG4P and 19.9% (20.5) for ENL ($P < 0.0001$, CI $\Delta = 5.8$ -8.7%, $n = 839$). During the CRC visit, MARD in the hypoglycaemic range (≤ 70 mg/dL) was 17.6% (12.2) for DG4P and 24.6% (18.8) for ENL ($p < 0.005$, CI $\Delta = 3.1$ -10.7%, $n = 117$). Both sensors showed higher MARD during hypoglycemia compared to euglycemia (70-180 mg/dL) (DG4P 17.6% vs. 13.0% and ENL 24.6% vs. 14.2%).

Conclusion: During circumstances of intended use, including both a CRC and home phase, the Veo Enlite sensor was noticeably less accurate than the G4 Platinum sensor. Both sensors showed lower accuracy in the hypoglycaemic range. DG4P was less affected by this negative effect of hypoglycemia on sensor accuracy than ENL.



Clinical Trial Registration Number: NCT01751932

Supported by: Dexcom, USA.

1020

How current continuous glucose monitoring (CGM) users translate CGM data into diabetes management: results of a survey

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Background and aims: The use of real time (RT) CGM devices is becoming the standard of care for treating patients with type 1 diabetes (T1D) as several studies have shown that children and adults that regularly use CGM improve their glycemic control. However, the specific interventions that patients make on a day-to-day basis in response to the CGM information have not been determined. This study was conducted to learn more about current CGM

practices and assess how CGM users respond to their CGM information in a real-world setting.

Materials and methods: A 70 question clinical scenario-based survey was developed that probed 5 topics: subject characteristics and general CGM use, treating and preventing incidental and mealtime hypo- and hyperglycaemia and nocturnal issues. The survey was extensively beta-tested to make sure questions were clearly written and unambiguous. Clinicians that actively prescribe CGM from across the US were contacted and asked to recruit and provide a survey web link to regular Dexcom CGM users (>6 days a week on average) from their practice.

Results: 222 of 300 respondents had T1D and are analyzed in this abstract: mean age (46 ± 14 years), duration of T1D (22 ± 14 years), self-reported HbA1c ($6.9\% \pm 0.8\%$), 52% male, 75% used CSII and 25% MDI (75% had used CGM >1 year), education- 65% university graduates or had another advanced degree. Key findings: The majority of the participants viewed the RT information (51%) or hypo/hyper alarms (30%) as the most important information; only 3.6% of subjects thought the computer downloads analysis was most helpful. To prevent hypoglycemia, 70% of participants reported they would prophylactically consume carbohydrates in response to a displayed glucose of 120mg/dl with a decreasing trend (angled or downward rate of change arrow). 42% of respondents stated that at least one time in the last 6 months, their CGM device alerted somebody around them to respond to their hypoglycemia alarm when they themselves were unable to. 70% and 66% of participants report waking up at night at least once per week in response to alerts for hypo- and hyperglycemia respectively. In a scenario-based question about response to a low alert waking them up at midnight, 50% of respondents stated they would treat the low glucose without confirming with their meter. 65% of participants reported that since starting CGM, the number of boluses they took per day had increased. Daytime and nighttime glucose targets commonly changed- 57% lowered their daytime glucose targets. Participants reported a mean increase in correction insulin dose (unrelated to a meal) for 1 arrow up was 111%, a mean decrease in the correction dose for 1 arrow down was 41%, relative to the correction dose with a flat arrow. For a 50 gram glucose meal at euglycemia, the meal dose increased by 81% for 2 arrows up and decreased by 53% for 2 arrows down, relative to the meal dose with a flat arrow. Most (59%) participants also reported they adjusted the insulin timing in each of the scenarios, based on the direction and rate of change.

Conclusion: Patients use CGM data in many of their clinical decisions and for multiple aspects of their diabetes management. These changes contribute to safer diabetes management and improved outcomes. The adjustments reported by patients in this survey to their mealtime and correctional insulin doses are much larger than current recommendations.

PS 085 Psychological distress

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Metabolic control in type 1 diabetic children with self report depression: 3-year follow-up

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Background and aims: Many studies show there is a higher risk of depression in patients with diabetes than in healthy population. Depression has been associated with worse disease outcomes and readmissions in adults with diabetes. Less is known about the effect of depression on metabolic control and hospital admissions in children and adolescents with type 1 diabetes. The aim of this study was to examine if depression is associated with worse metabolic control and repeat hospitalisations in children and adolescents with type 1 diabetes in 3-year follow-up.

Materials and methods: In years 2010-2012 we screened 477 children for depression: during the routine visit in the outpatient clinic 252 girls and 225 boys, with diabetes duration > 1 year filled in Polish version of Children's Depression Inventory by M. Kovac, a self-report questionnaire consisting of 27 items. At the same time other data was collected: sex, age, diabetes duration, HbA1c, BMI, daily insulin dose. Medical parameters of 139 children (65 boys, 74 girls, age 11.38 ± 1.85 diabetes duration 4.53 ± 3.05 , ins/kg 0.81 ± 0.20 , BMI 19.18 ± 0.2 , HbA1c 7.5 ± 1.3 at baseline), collected for 3 years were analysed.

Results: 5% (22/137 participants scored ≥ 13 , indicating elevated depressive symptoms. There was no difference between children with CDI ≥ 13 and CDI < 13 in diabetes duration ($p = 0.483$) and age ($p = 0.329$). We found significant relationship between scores in CDI and HbA1c in subsequent years of follow up: 2010 - $r = 0.1753$ $p = 0.039$, 2011 - $r = 0.1349$ $p = 0.037$ and 2013 - $r = 0.2629$ $p = 0.0018$ but not 2012 - $r = 0.1159$ $p = 0.183$. CDI scores were not associated with daily insulin dose for all years but the baseline (2010 $r = 0.181$, $p = 0.033$, 2011 $r = 0.139$ $p = 0.125$, 2012 $r = 0.099$, $p = 0.273$). We didn't find the difference between participants with CDI ≥ 13 and CDI < 13 in HbA1c ($p = 0.573$), daily insulin dose ($p = 0.113$) or BMI ($p = 0.541$). At the same time children with CDI ≥ 13 had higher insulin dose/kg/d ($p = 0.024$). Surprisingly we didn't find the difference between children with CDI ≥ 13 and CDI < 13 in the number of hospitalisations ($p = 0.797$) or psychological care ($p = 0.064$).

Conclusion: 15% participants show elevated depressive symptoms as assessed with Children's Depression Inventory at the baseline. Children and adolescents with higher scores on the CDI don't differ from patients without depressive symptoms in age, BMI and daily insulin dose. Although depressive symptoms are associated with worse metabolic control children with higher scores in CDI achieve the same HbA1c level as their peers with lower CDI scores. This trend is seen for the whole period of follow-up. Our results show that depressive symptoms are not associated with higher use of hospital facilities. Still it is necessary to pay attention to emotional wellbeing of children and adolescents with diabetes type 1 regardless of HbA1c.

1022

The assessment of factors related to depressive symptoms in adult patients with type 1 diabetes

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Background and aims: Depressive disorders are more common among people with diabetes compared with general population. The presence of depressive symptoms may reduce motivation and the treatment adherence and in fact worsen the treatment outcomes. The aetiology of depression is complex and not only related to diagnosed chronic disease. The aim of this study was to assess factors related to depressive symptoms in adult patients with type 1 diabetes.

Materials and methods: 304 subjects (164 women), aged 43 (33-52) years, type 1 diabetes duration 26 (33-31) years, HbA_{1c} 7.8 (7.1-8.8)% were included. Subjects diagnosed with chronic complications causing disability (blindness, end stage renal disease, painful peripheral neuropathy, limb amputation) were excluded to avoid the presumable impact of irreversible dis-

ability on questionnaire answers. Depressive symptoms were assessed using Beck Depression Inventory (BDI). Additionally, Problem Areas in Diabetes Questionnaire (PAID), Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) were completed. Data on social, economic and lifestyle factors were collected. Metabolic control expressed by HbA_{1c} and medical history was assessed via medical chart review. The studied group was divided into two subgroups according to the presence or absence of depressive symptoms based on the BDI standard cut-offs.

Results: 41% of study participants presented any depressive symptoms. They were more frequent among women ($p<0.0001$), less educated, unemployed and not physically active participants ($p=0.016$, $p=0.015$, $p=0.0014$ respectively). Fear of hypoglycaemia and diagnosed chronic complications of diabetes appeared more frequently among patients exhibiting depressive symptoms ($p=0.013$, $p=0.04$ respectively). The intensity of depressive symptoms correlated with fatigue as well as with degree of coping with diabetes ($r=0.56$, $p<0.0001$; $r=0.42$, $p<0.0001$ respectively). No relation of metabolic control (HbA_{1c}) with depressive symptoms was demonstrated.

Conclusion: In adult type 1 diabetic patients depressive symptoms are related not only to chronic disease, but also to sex, socioeconomic status and lifestyle. The results of this study may be useful in delineating the complex treatment plan including nonpharmacological and psychological interventions.

Supported by: Poznan University of Medical Sciences

1023

The effect of a diabetes-specific cognitive behavioural treatment programme (DIAMOS) for people with diabetes and subthreshold depression

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Background and aims: Subthreshold depression is one of the most frequent mental comorbidities in people with diabetes and is associated with a poorer long-term prognosis. Since specific intervention concepts are missing a new self-management oriented group programme (DIAMOS) was developed for this patient group and evaluated in a randomised trial.

Materials and methods: The active control group (CG) received diabetes education. DIAMOS consisted of cognitive behavioural interventions aiming at the reduction of diabetes distress. Patients completed several questionnaires at baseline and follow-up: The Center of Epidemiological Studies-Depression Scale (CES-D), the Patient Health Questionnaire (PHQ 9), the Problem Areas in Diabetes Questionnaire (PAID) and the Diabetes Distress Scale (DDS). Primary outcome was the reduction of depressive symptoms. Secondary outcomes were diabetes distress, well-being, self-care behaviour, diabetes acceptance, diabetes treatment satisfaction, HbA_{1c} and inflammatory markers. 214 participants were randomised.

Results: Baseline characteristics (age 43.3 ± 13.3 yrs., female gender 56.5%, diabetes duration 14.2 ± 10.5 yrs., type 2 diabetes 34.1%, BMI 28.7 ± 7.1 kg/m²) were comparable between both interventions groups except BMI and diabetes type. At 12-month follow-up there was a significant greater reduction of the CES-D- and PHQ 9-scores in DIAMOS compared to the CG (Δ -3.7, 95%-CI 0.57 to 6.85 $p=.021$ respectively Δ -1.49, 95%-CI 0.08 to 2.90; $p=.039$). The risk of incident major depression was reduced (OR 0.63, 95%-CI 0.42 to 0.96, $p=.028$) Also PAID-scores (Δ -8.3 95%-CI 3.33 to 12.72, $p=.001$) and DDS-scores (Δ -0.22 95%-CI 0.02 to 0.42, $p=.042$) were significantly reduced. C-reactive protein was significantly more lowered (Δ -0.25 95%-CI 0.02 to 0.48 $p=.035$). No effect of the intervention was observed in other inflammatory markers (IL1RA, IL6 and adiponectin).

Conclusion: DIAMOS is more effective in lowering depressive symptoms and diabetes related distress in diabetic patients with subthreshold depression. DIAMOS also has a preventive effect regarding the incidence of major depression.

Clinical Trial Registration Number: NCT01009138

Supported by: the BMBF within the competence network Diabetes

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Non severe hypoglycaemia does not affect the prevalence of diabetes related stress

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Background and aims: The occurrence of hypoglycaemia is supposed to be associated with lower quality of life in patients with type-2 diabetes. Furthermore hypoglycaemia is suspected to be a trigger for depression. We investigated the association of hypoglycemic events with diabetes related stress in a large outpatient cohort with diabetes type 1 (DM1) and type 2 (DM2).

Materials and methods: In a cross-sectional study we assessed hypoglycaemia and diabetes related stress with the PAID questionnaire in 785 people with diabetes in an university outpatient department for metabolic diseases over a period of three months (1.11.2012 to 31.01.2013). A PAID score ≥ 40 indicates a depressed mood. 193 people (24.6%) had DM1 (HbA_{1c} 7.2%, age 54.5J, diabetes duration 22.3J, BMI 27.0kg/m²), 182 (23.2%) had DM2 without insulin therapy (HbA_{1c} 6.6%, age 63.5J, diabetes duration 8.6J, BMI 31.1kg/m²) and 410 (52.2%) had DM2 with insulin therapy (HbA_{1c} 7.1%, age 68.0J, diabetes duration 18.6J, BMI 33.6kg/m²). Patients with and without hypoglycaemia were compared. Mild hypoglycaemia was defined as a condition with typical symptoms (e.g. sweating, impaired concentration) disappearing quickly after carbohydrate ingestion or plasma glucose <2.7 mmol/l with or without typical symptoms. Severe hypoglycaemia was diagnosed if intravenous glucose injection or intramuscular glucagon injection was necessary. HbA_{1c} was adjusted according to the mean normal value of healthy people in the DCC trial (5.05%).

Results: People with DM1 had 1.09 mild hypoglycaemia/week and 0.04 severe hypoglycaemia/last 12 months. There were no significant differences in diabetes related stress comparing people with DM1 with ($n=111$) and without ($n=82$) mild hypoglycaemia (PAID score 19.0 ± 14.6 vs. 16.5 ± 14.6 , $p=0.284$), as well as with ($n=7$) and without ($n=186$) severe hypoglycaemia (20.7 ± 18.0 vs. 18.1 ± 15.8 , $p=0.666$). The mean PAID score of both groups is well below the threshold for depressive mood (PAID score <40). The frequency of mild hypoglycaemia/week in people with DM2 without and with insulin therapy is low (without insulin 0.03, with insulin 0.1). People with DM2 treated without insulin with ($n=4$) and without ($n=178$) mild hypoglycaemia have no different PAID score (20.3 ± 15.0 vs. 15.5 ± 14.5 , $p=0.515$). People with DM2 on insulin with ($n=33$) and without ($n=377$) mild hypoglycaemia as well show no differences in the PAID score (16.7 ± 11.2 vs. 16.9 ± 14.8 , $p=0.945$). Severe hypoglycaemia/last 12 months did not occur in people with DM2 in this study group.

Conclusion: In an outpatient setting neither in people with diabetes type-1 nor type-2 mild and severe hypoglycaemia are associated with increased diabetes related stress or increased incidence of depressive mood.

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Psychosocial distress in a clinical setting: Is PAID 1 a valuable tool to disclose distress

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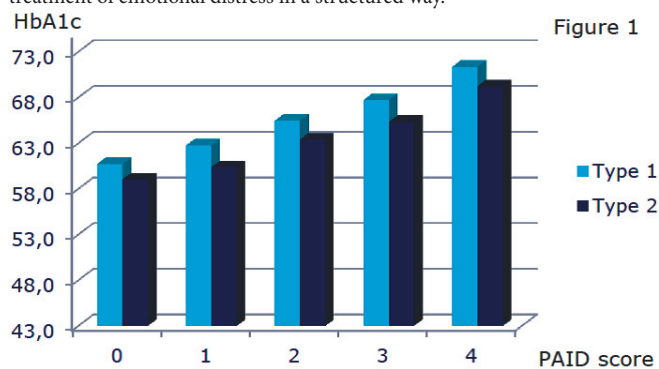
Background and aims: Psychosocial problems are common among patients with diabetes and often not recognized and addressed in the relatively short and busy meeting with the health care professional in daily clinical setting. Problem Areas in Diabetes (PAID) 1 (question 12: 'Worrying about the future and the possibility of serious complications') has been identified as a one-item screening tool, with high sensitivity and specificity for the recognition of diabetes-related emotional distress. In a specialized diabetes clinic we have in 2011 implemented PAID 1 at the biennial status examination performed by the diabetes nurse to evaluate whether this is a feasible screening tool in the daily clinical setting.

Materials and methods: 3274 patients, aged 54.7 ± 42.3 years, male 56.6 %, Type 1 diabetes 67 %, disease duration 22.8 (0-80) years, are included in this material. The result of the screening is registered in the Electronic Patient File from where data is worked up. PAID is a questionnaire for self-administration developed to explore and screening for diabetes-related emotional issues from the patient's perspective. The table is built on a 5 point Likert scale from a point „not a problem“ to the „serious problem“.

Results: 41 % type 1 and 36 % type 2 diabetes patients show significant distress (PAID 1 score >2), not dependent on diabetes duration, confirming data from previous PAID studies. No influence of gender was seen. A previous

study has found PAID score to be weakly related to HbA1c. In our study, PAID 1 score is also related to poor metabolic control, figure 1.

Conclusion: We conclude that PAID 1 is suitable as a rapid tool to disclose emotional distress in a busy clinical setting. PAID 1 has been easily implemented by the nurses and it is found valuable as an opening question in the consultation in order to discuss emotional distress and worries related to diabetes treatment, complications and daily life. Next step will be to study change in PAID 1 over time and how we can manage further screening and treatment of emotional distress in a structured way.



1026

Associations between glycaemic control, depressed mood, clinical depression and diabetes distress

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Background and aims: Although depression has been associated with poor glycemic control in patients with type 2 diabetes (T2DM), the available data are inconsistent and both methodological approaches and data quality vary significantly across studies. Specifically, depression has been defined and measured in multiple ways, including identification of a clinical syndrome, use of a depression rating scale and as measured by distress linked to diabetes and its management. This exploratory, post hoc analysis assessed the association between depression, evaluated by these 3 methods, and glycemic control over 2 years in patients with T2DM initiating insulin in a single treatment trial.

Materials and methods: We analyzed data from a 24-month, prospective, observational study that evaluated glycemic response in patients with T2DM who initiated insulin therapy (N=985) in 5 European countries. Secondary measures included patient-reported diagnosis of depression at baseline, severity of depressed/anxious mood (EuroQol (EQ)-5D item) and diabetes-related distress (Psychological Distress domain of the Diabetes Health Profile, DHP-18). The latter two measures were assessed at baseline and 5 time points throughout the study. Glycemic control was measured by A1c at these same time points. Analyses employed t-tests to assess the unadjusted baseline difference in A1c between patients with and without the respective depression parameter. The potential effect of demographic and clinical confounding variables was controlled through a linear model structure.

Results: At baseline, A1c of patients with depressed mood (moderately or extremely depressed/anxious) was higher, but not statistically significantly different from patients without depressed mood (9.6% vs. 9.5%, respectively, $p=.303$). However, patients with depressed mood had higher A1c values throughout the 24 months follow up ($p<.001$). Patients with clinical depression had a higher baseline A1c than patients without clinical depression (10.8% vs. 9.4%, respectively, $p<.001$) and higher A1c values during the first 6 months ($p<.010$) following insulin initiation. Significant group differences were not observed at subsequent time points. Patients with high diabetes distress had higher A1c at baseline as compared to patients with low distress (9.9% vs. 9.4%, respectively, $p<.001$) and higher A1c values were observed in the high distress group throughout the 24 months follow up ($p<.001$). Reported results are based on the unadjusted group comparisons.

Conclusion: In this exploratory, post hoc analysis we demonstrated consistent and significant associations between poorer glycemic control and each of the 3 depression parameters: depressed mood, clinical depression and diabetes distress. The associations with poorer A1c were observed concurrently at baseline for clinical depression and diabetes distress and longitudinally for

all 3 depression parameters. Our findings support - in this patient group - the previously reported association between depression and diabetes control. However, this analysis was not designed to assess causality and will require replication. The similar, but not identical findings, of the 3 unique depression measures suggest that each tool may capture a different but interrelated condition that may require assessment and management to optimize glucose control in patients with T2DM.

Supported by: Eli Lilly and Company

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Association of the presence and severity of depression with demographic, socioeconomic, glycaemic and metabolic risk factors in type 2 diabetes

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Background and aims: Depression is a serious mental disease quite prevalent. Surveys have shown a strong association between depression and diabetes mellitus with poor outcomes in disease control. The purpose of this study is to explore possible correlations between demographic, socioeconomic and metabolic parameters with the presence and severity of depression in people with type 2 diabetes.

Materials and methods: 269 patients (132 males, aged 65 ± 10.2 years) with type 2 diabetes were included in the study. Information regarding demographic characteristics, socioeconomic status, anthropometric and metabolic parameters, glycemic control, type of antidiabetic treatment and the presence of complications of diabetes were collected from all patients. The questionnaire scale BDI-II is widely used for depression and consists of 21 questions each answer being scored on a scale value of 0 to 3. The total score ranges from 0 to 63. Higher total scores indicate more severe depressive symptoms. Statistical analysis was performed using STATA 9.0 software.

Results: Severity of depression showed a statistically significant relationship with the following parameters: age (Coefficient=-0.21, $p<0.0001$), living with their children (Coefficient=4.6, $p=0.012$), menopause (Coefficient=-10.3, $p=0.039$), bmi (Coefficient=0.53, $p=0.001$), HbA1c (Coefficient=1.68, $p=0.002$), oral hypoglycemic agents (Coefficient=-5.5, $p=0.035$) and insulin (Coefficient=5.5, $p=0.006$). On multivariate analysis, HbA1c showed significant positive association with the severity of depression (Coefficient = 1.73, 95% CI: 0.42 - 3.04, $p = 0.010$). Moreover, analysis regarding glycemic control revealed that participants with HbA1c < 7% had lower BDI-II score than those with HbA1c > 7% ($p=0.023$).

Conclusion: Higher HbA1c levels appear to act favorably to the presence and severity of depression. Furthermore, good glycemic control is inversely associated with depression in people with type 2 diabetes.

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Improvement in depressive symptoms is associated with reduced markers of inflammation and oxidative damage in type 2 diabetic patients

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Background and aims: Processes of inflammation and oxidative damage in diabetic patients suffering from subsyndromal depression have not been sufficiently clarified. This study was aimed at examining one-year changes in inflammatory and pro-oxidative biomarkers in patients treated for elevated depressive symptoms that did not reach criteria for clinical depression.

Materials and methods: A randomized controlled comparison of two behavioural interventions for subsyndromal depression - six-week psychoeducation and physical exercise, and a control condition consisting of brief diabetes re-education - was performed in a sample of 209 type 2 diabetic patients with subsyndromal depression (aged 57 ± 6 yrs, 54% female, with diabetes duration of 11 ± 8 yrs, 32% insulin treated, with HbA1c of 7.3 ± 1.2 [56 ± 12.3 mmol/mol] and BMI of 30 ± 5 kg/m²). Clinical depression was excluded by using a Structured Clinical Interview for the DSM-IV Axis I disorders. A level of depressive symptoms, endogenous inflammatory markers (leukocytes, CRP), indicators of antioxidant capacity (myeloperoxidase index [MPXI], plasma

uric acid), and markers of oxidative damage (sialic acid, urinary-8-OH-deoxyguanosine [u-8-OH-dG]) were assessed at baseline, and after six- and twelve months. Repeated measures ANOVAs were employed to determine within- and between-group differences in depressive symptoms and measured biomarkers at follow-ups.

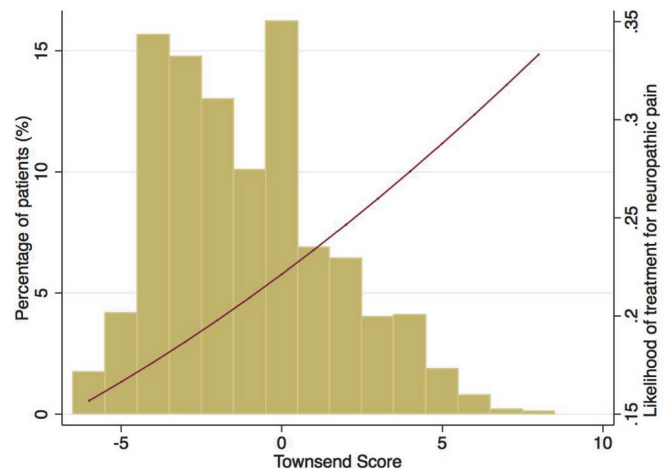
Results: Participants in the psychoeducational, physical exercise and the control group improved equally in depressive symptoms from baseline to 12-month follow-up (time versus time x group effect; $F=12.51$ $p<.0001$ $\eta^2=0.07$ and $F=0.609$ $p=0.656$ $\eta^2=0.007$, respectively). Total leukocytes significantly decreased in all three groups (7.7 ± 1.8 to 7.1 ± 1.6 $F=15.72$ $p<.0001$ $\eta^2=.119$) as did sialic acid (2.1 ± 0.3 to 1.8 ± 0.3 $F=135.67$ $p<.0001$ $\eta^2=0.323$) and u-8-OH-dG (1.1 ± 0.5 to 0.99 ± 0.5 $F=10.66$ $p<.0001$ $\eta^2=0.06$). No within- and between-group changes were observed with respect to CRP and MPXI. Uric acid increased significantly during one year in all three groups (295.1 ± 84.9 to 315.8 ± 84.4 $F=12.53$ $p<.0001$ $\eta^2=0.07$), with time effects being greater in women in comparison with men ($\eta^2=0.13$ versus $\eta^2=0.03$).

Conclusion: Lowering depressive symptoms in subsyndromally depressed type 2 diabetic patients was found to be associated with long-term improvements in biomarkers of inflammation and oxidative stress. Treatment of even mild depressive symptoms may have favourable clinical implications.

Clinical Trial Registration Number: ISRCTN05673017

Supported by: EFSD

Conclusion: A higher level of socioeconomic deprivation seemingly may predispose to severe neuropathic pain in diabetes requiring pharmacological intervention. Targeted allocation of healthcare resources to this group may offer significant clinical benefits.



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Socioeconomic deprivation independently predicts symptomatic painful diabetic neuropathy in type 2 diabetes

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Background and aims: Painful peripheral neuropathy in people with type 2 diabetes is a disabling complication. We explored associations of this condition with socioeconomic deprivation in an area of the UK, Cheshire which has a wide variation in demographic profile.

Materials and methods: The Townsend index of socioeconomic deprivation was examined in the pseudonymised GP records of 15388 (44.1% female) individuals with type 2 diabetes in Cheshire UK, and related to prevalence of drug treated painful diabetic neuropathy. We also analysed prescription trends with respect to pharmacotherapy for neuropathy pain relief.

Results: Treatment for neuropathic pain was initiated in 3266 (21.2%) of patients. Those on treatment were older [68.2 (95% CI 67.8-68.7) vs 66.6 (66.4-66.8) years] than those not on treatment. There was no difference in HbA1c (7%, 55 mmol/mol). The odds of having treated painful peripheral neuropathy increased by 3.8 % for each 5 years of increasing age of the patient odds ratio (OR) 1.05: 95% confidence interval (CI) 1.04-1.07; $p<0.0001$. There was a 3% increased chance for each unit increase in BMI OR 1.03: 95% CI 1.02-1.03; $p<0.0001$, but no relation with HbA1C. (OR 1.0: 95% CI 0.99-1.0; $p=0.58$). There were significant differences between the groups for the Townsend deprivation index, with a greater proportion (30.6% vs 22.8% of patients with treated neuropathic pain) having a score of ≥ 1 ($X^2=83.9$, $p<0.0001$). Multivariate logistic regression analyses indicated that each unit increment in the Townsend index was associated with an 6% increased odds of requiring neuropathic pain treatment [odds ratio (95%CI) 1.06 (1.05-1.08), $p<0.0001$] independent of five year age band, BMI, gender, systolic BP, eGFR, HbA1C and total cholesterol.

PS 086 Psychology and quality of life

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Physicians' challenges when discussing the type 2 diabetes diagnosis with patients: insights from a cross-national study (IntroDia™)

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Background and aims: Physician communication at diagnosis of type 2 diabetes (T2D) may impact patient self-care, quality of life, and outcomes. IntroDia™ is a survey of ~17000 T2D patients and physicians in 26 countries to investigate early physician-patient communication and its potential consequences.

Materials and methods: As part of IntroDia™, we surveyed 6753 physicians from Asia, Europe, America, Africa, and Australia using an online survey that included a novel questionnaire of 12 challenges that physicians may encounter at T2D diagnosis, and the Jefferson Scale of Physician Empathy.

Results: Across countries, 76–100% of physicians (88% overall) agreed that conversations with patients at T2D diagnosis impact on patients' disease acceptance and treatment adherence. 92% of physicians wanted tools to help patients sustain behavioural change. Using factor analysis, the 12 items resulted in loading onto 2 factors (Table): Discouraged with Patients at Diagnosis (DPD; $\alpha = 0.87$), comprising 8 related challenges, and Frustrated with Situation at Diagnosis (FSD; $\alpha = 0.72$), comprising 4 challenges. Correlation between these 2 factors suggested related, but distinct, groups of challenges ($r = 0.64$, $p < 0.0001$). Factor scores varied globally (DPD highest in France; FSD in Japan). Upon adjusting for demographic/clinical practice variables, regression models showed a negative relationship between physician empathy and perceived challenges for total score (all 12 items) as well as DPD and FSD (all $p < 0.0001$).

Conclusion: Many physicians, especially those scoring lower on empathy, report significant challenges and frustrations during conversations with patients at diagnosis of T2D. Most physicians wanted tools to help patients sustain behavioural change. Supporting use of empathy-related skills may contribute to better patient outcomes.

12-item Questionnaire: "Challenges at Diagnosis"						
How often are you encountering the challenges described by these statements during your diagnosis conversations?	% physicians answering In ... diagnosis conversations (n=6753)					Factor
	...no	...a few	...some	...most	...all	
Shortly after diagnosis, patients fail to keep up with the required behavioural changes and return to old habits	3	22	47	26	2	DPD
The patients do not understand the seriousness of the situation	4	25	48	21	2	DPD
It is frustrating to work with T2D patients that don't follow my recommendations	14	34	33	15	5	DPD
It is difficult to convince patients that they can take control of their health	7	33	42	16	2	DPD
It is difficult to develop a treatment plan with patients that they will follow	11	39	36	13	2	DPD
It is difficult to convince patients to stay positive	9	41	37	12	2	DPD
Patients do not see the benefits/need to collaborate with me to manage the disease	12	42	34	10	2	DPD
Patients leave the visit without having a clear idea of what they are supposed to do	20	43	26	9	1	DPD
I don't have enough time	25	25	27	17	6	FSD
I do not receive enough support from others (my team, nurses, etc.)	49	25	15	8	3	FSD
It is difficult to deal with patients' emotional responses to the diagnosis	17	41	31	9	1	FSD
It is difficult to explain diabetes to these patients	25	38	27	8	2	FSD

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Health-related quality of life and the relation to metabolic syndrome, type 2 diabetes, degree of obesity and low-grade inflammation in obese individuals

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Background and aims: We assessed the differences in health-related quality of life (HRQL) in obese participants with and without metabolic syndrome (MetS) and type 2 diabetes (T2D), by degree of obesity and level of inflammation (high sensitive C-reactive protein (hsCRP)). HsCRP is related to Obesity, MetS and T2D.

Materials and methods: Participants were derived from the random population-based LifeLines study in the Netherlands. In total, 53,266 subjects (39,540 normal weight and 13,726 obese; BMI $>30 \text{ kg/m}^2$), aged 18–80 years, were included. Subjects with diabetes were those with known T2D or had a fasting blood glucose $>7.0 \text{ mmol/L}$. HRQL was assessed with the Short Form-36. Sex-corrected physical (PCS) and mental component score (MCS) were calculated. MetS was measured with the NCEP ATP III (2005). ANCOVA was used to compare the PCS and MCS score, with age as covariate. Clinically relevant differences in PCS and MCS were defined as a minimum difference score of respectively, 2 and 3 points between groups of patients. We defined a poor outcome on individual scales of the PCS and MCS as a score below 80 points. Results on individual scales were stratified by gender.

Results: PCS and MCS: Compared to normal weight individuals, obese subjects had a significantly lower PCS (ranging from 3.1–7.5 points difference), pending on their MetS and T2D status, degree of obesity and level of inflammation. However, no clinically relevant changes were found for MCS. Obese subjects with MetS had a significant lower PCS (49.7 vs. 51.1) and MCS (50.4 vs. 51.2) than obese subjects without MetS, however these differences were small. Having T2D only affected the PCS (50.7 vs. 47.5; $p < 0.001$). Increasing BMI was associated with a worse score of both PCS and MCS. However, test for linearity was only significant for PCS ($p < 0.001$). Subjects with elevated hsCRP had a lower PCS than those with normal hsCRP (test for linearity, $p < 0.001$). Individual scales: Among obese males, MetS affected physical functioning, role physical and general health within the physical health domain, compared to obese men without MetS (all $p < 0.01$). Within the dimension mental health, only social functioning and role emotional was affected by MetS. In obese women all scales of the physical and mental health domain was affected by MetS. Higher BMI among the obese subjects, was associated with having a poor outcome on all scales (test for linearity, all $p < 0.01$), except for role emotional in men and mental health in women. Elevated hsCRP showed more affected scores on scales within the dimension of physical health, than within the dimension of mental health. A higher percentage of obese male and female subjects with T2D had a worse score on all scales of physical health compared to those without T2D. Only the score on social functioning was affected by T2D ($p < 0.01$).

Conclusion: Obese individuals had a lower HRQL compared to normal weight individuals, even in the absence of MetS, T2D or inflammation. Having T2D, a BMI $\geq 40 \text{ kg/m}^2$ or hsCRP $>10 \text{ mg/L}$ was associated with clinically relevant decreased physical health. MetS, T2D, increasing BMI and low-grade inflammation were all associated with poorer outcome of scales in the domain of physical health rather than in the mental health domain.

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Day napping and risk of type 2 diabetes: a dose-response meta-analysis

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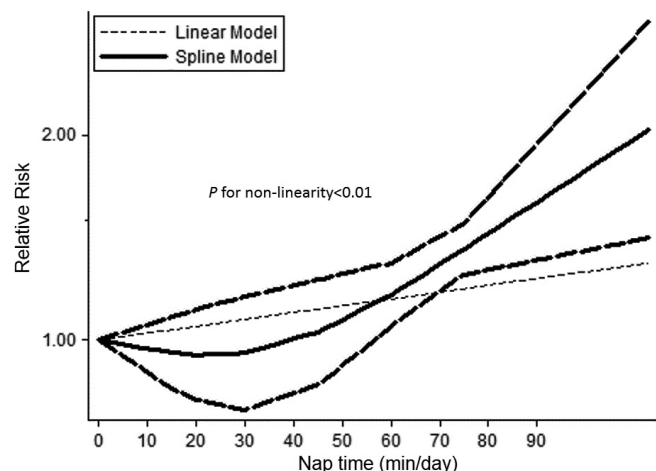
Background and aims: A nap is a short period of sleep, typically during daylight hours, and the habit of napping is widely prevalent around the world. Daytime naps are usually brief, but can range from a few minutes to a few hours. The frequency varies from taking an occasional nap to planned rest periods several times daily for habitual nappers. Some individuals take a nap because they are excessively sleepy during the daytime as a result of a sleep disorder. We performed a meta-analysis of evidence on the association between napping and the risk of type 2 diabetes and attempted to quantify the potential dose-response relation.

Materials and methods: We searched Medline, the Cochrane Library, Web of Science, and Science Direct for articles published up to March 2014 using the keywords nap (siesta) and diabetes. We also searched the references of

the original studies that we identified. Observational studies reporting risk estimates for type 2 diabetes were assessed. To ascertain the validity of the eligible studies, the quality of each report was appraised with reference to the STROBE statement. In addition, the Newcastle-Ottawa Scale for assessing the quality of prospective cohort studies in meta-analyses was used to quantify the validity of each study. The adjusted relative risk and 95% confidence interval were calculated with the random effect model. Dose-response relations were evaluated using data from different nap categories in each study. Two authors independently confirmed the eligibility of the studies and collated the data. Any discrepancies were resolved through discussion. Sex was employed as an independent category for comparison. Publication bias was evaluated by the funnel plot, Begg test, and Egger test.

Results: Among 1,035 potential studies, 225,717 Asian and Western subjects stratified into 5 categories were identified. The analyses performed in each study were well adjusted for several confounders for diabetes. Pooled analysis revealed that a longer nap time (≥ 60 min/day) significantly increased the risk of type 2 diabetes (relative risk 1.46 (1.23–1.74, $p < 0.001$)), while shorter nap time (< 60 min/day) did not (0.95 (0.75–1.21, $p = 0.68$)). Meta-analysis showed a significant non-linear dose-response relation between nap time and the risk of diabetes (P for non-linearity < 0.01) (Figure 1), with no effect of nap time up to about 40 minutes/day followed by a sharp increase in the risk of diabetes at longer times. Publication bias was not significant.

Conclusion: There was a J-curve relation between nap time and the risk of type 2 diabetes, with longer nap times being associated with an increased risk.



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Longterm impact of childhood onset type 1 diabetes: social insertion, quality of life, sexuality

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Background and aims: Little is known about the long-term social outcome of diabetic children. This study aimed to describe socio-professional life, quality of life (QOL) and sexuality at adult age of children and adolescents with type 1 diabetes (T1D).

Materials and methods: Of 721 adults with childhood-onset T1D identified in the nationwide French Register of Incidence of Diabetes, 388 (53.8%; age = 28.5 ± 3.1 yrs; age at diagnosis of diabetes = 11.5 ± 2.5 yrs; duration of diabetes = 17.0 ± 2.7 yrs) responded to a questionnaire (198 items). Data were compared to French general population (FGP) using indirect standardized ratios (SIR) matched for age, gender, period +/- educational level and familial life, or Z-scores adjusted for age, gender and period. Linear regression was used to investigate correlates of Physical (PCS) and Mental (MCS) SF36 Composite Scores.

Results: Educational level was similar to FGP (68.6% \geq high school degree; SIR = 1.06 (CI95%: 0.93; 1.20)), whereas school delay in first year of primary school was lower (5.3%, SIR = 0.49 (0.30; 0.75)). Unemployment was increased in women (15.3%, SIR = 1.50 (1.0; 2.1)) but not in men (8.6%, SIR = 0.96 (0.51; 1.57)). Professional occupation and employment contract patterns were close to what was expected. Adults with T1D were less likely

to be homeowner (25.7%, SIR = 0.72 (0.57; 0.89)). Frequency of daily alcohol consumption was higher than in FGP (SIR: men = 3.34 (2.38; 4.54); women = 6.53 (4.57; 12.99)); 7.2% (12/166) of men consumed more than recommended (2 glasses per day), 5.0% (11/220) of women (1 glass per day). Familial life pattern was not different from FGP and among those who planned to have children, 72.2% (125/173) had one or more biological child. Overall, 7.1% were very dissatisfied with sexual life (SIR = 1.90 (1.18; 2.75)). Sexual problems prevalence was higher in women (SIR: dysorgasmia = 1.91 (1.21; 2.88); decrease/loss of desire = 2.11 (1.35; 3.08)), but similar in men; Social discrimination was more frequent (SIR = 5.64 (4.64; 6.62)). PCS and MCS were respectively moderately (mean = 52.0 ± 7.5 ; mean Z-score = -0.2 (CI95%: -0.3; -0.1)) and substantially altered (mean = 42.1 ± 12.4 ; mean Z-score = -0.7 (-0.8; -0.6)). Fatigue and abandoning sport were associated with lower PCS and MCS; educational level and presence of diabetes complication were also predictive of worse PCS, and sexual dissatisfaction and negative feelings related to transition pediatric/adult care were predictive of lower MCS. Participants with at least one vascular complication (n = 136) had preserved social outcomes but decreased PCS (mean = 50.0 ± 8.3 ; mean Z-score = -0.5 (-0.7; -0.3)) and MCS (mean = 40.3 ± 12.3 ; mean Z-score = -0.9 (-1.1; -0.7)).

Conclusion: Young adults with T1D have a satisfying social well-being but alteration of MCS, frequent dissatisfaction with sexuality and uncommon alcohol consumption suggest the high impact of disease on morale, especially in women. Improving the screening of sexual problems and alcohol consumption, as well as optimize the psychological support of patients to cope with the T1D burden must be high priorities for health caregivers.

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Spectrum of diabetes research does not reflect patients' scientific preferences

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Background and aims: Involvement of patients and their relatives in planning of research has been proposed to increase the patient orientated relevance of scientific investigations. Here we compare the proportions of research topics patients would like to see and the trend in the distribution of research categories of accepted EASD abstracts during the last four years.

Materials and methods: We evaluated the spectrum of diabetes research as published during the EASD meetings 2010 - 2013 and compared them with the results of a survey in 918 lay people (652 patients with diabetes, 205 relatives, 61 others concerned) interested in diabetes research, who had described their research preferences in diabetes. To obtain a fairly unselected sample of an interested lay group a questionnaire was published in a popular weekly news magazine inviting patients with diabetes and their relatives to state which area of research was of top priority to them. Nine research categories had been defined by consensus by four scientifically active diabetologists, and their completeness had been checked by testing whether all EASD abstracts published 2008 were covered by a defined scientific field. For this 4-year evaluation two reviewers (SA and PTS) independently assigned each EASD abstract to one of the nine research categories and also classified them according to a commercial sponsorship as indicated in the abstract EASD booklet. The 4-year period was analyzed in terms of changes or trends observed in the proportions of EASD research topics and the results were compared with patients' preferences.

Results: "Development, pathophysiology and prevention of diabetes" was the primary category of scientific interest for 25% of patients, 19% preferred "transplantation and cell therapy" and 16% "blood glucose measurement, devices and artificial pancreas". During the 4 years of EASD abstract investigation 50% or more of total research activities was constantly dedicated to "development, pathophysiology and prevention of diabetes", less than 2% to "transplantation and cell therapy" and 2.8% to 4.3% to "blood glucose measurement, devices and artificial pancreas". The average proportion of EASD research related to "blood-glucose lowering therapy without insulin" and "insulin therapy" more or less corresponded with the proportion of research patients would like to see (12.0% vs. 12.5%, and 6.9% vs. 4.7%, respectively). The majority of this research, however, was commercially funded. In total, non-commercial research was not closer correlated to patients' preferences compared to commercial research.

Conclusion: The distribution of diabetes research topics over the last four years as measured by the distribution of accepted EASD abstracts does not reflect what patients with diabetes and their relatives want to have investigated.

Better patient involvement and a reassessment of non-commercial funding strategies might help create more valuable research at least from patients' point of view.

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Glycaemic perception and other factors influencing adherence to self-monitoring blood glucose in patients with type 1 or type 2 diabetes

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Background and aims: Self-monitoring blood glucose is an important tool to achieve good glycemic control. However, many patients measure their glucose concentrations less often than recommended. This study was conducted to assess the factors associated with adherence to self-monitoring blood glucose in patients with diabetes mellitus treated with insulin.

Materials and methods: In this observational, prospective and multicenter study in patients with type 1 or type 2 diabetes treated with insulin as per usual clinical practice, socio-demographic and clinical characteristics were collected at the baseline visit. Glycemic perception was assessed through 4 monthly telephone calls asking patients about their perceived glycemic levels and then comparing these perceptions with their real values from the glucometer. A patient was considered adherent if the minimum number of glucose measurements established by the Spanish Society of Diabetes was carried out. To assess factors associated with adherence to self-monitoring blood glucose, a multivariate logistic regression was performed.

Results: A total of 2,700 patients were recruited, of whom 2,029 (75%) were considered valid for the analysis. With an age range of 18 to 80 years, the mean duration of the disease was 16.5±10.5 years and 30% of patients had type 1 diabetes. The majority of patients (81%) had an unsatisfactory glycemic control (baseline HbA1c ≥7%), which was significantly associated with an incorrect glycemic perception. Only 17% of patients had a correct glycemic perception. More than half of the patients (54%) were adherent to the self-monitoring according to the SED rules (95%CI: 52%, 56%), this being 51% in patients with type 1 diabetes. In patients with an age range of 18 to 44 years, the recommendation of >21 measurements/week (OR: 0.031; 95%CI: 0.015, 0.062) and alcohol consumption (OR: 0.452; 95%CI: 0.254, 0.805) were associated to low adherence. In patients with an age range of 45 to 64 years, an increase in the number of insulin injections/day was associated to a higher adherence (OR: 1.296; 95%CI: 1.073, 1.566), while the recommendation of >21 measurements/week (OR: 0.070; 95%CI: 0.048, 0.104) and the belief that it was possible to know their glucose levels without the glucometer (OR: 0.925; 95%CI: 0.877, 0.976) were associated to low adherence. In patients over 65 years, the recommendation of >21 measurements/week (OR: 0.112; 95%CI: 0.076, 0.163), obtaining the test strips from the health centre vs from the pharmacy (OR: 0.554; 95%CI: 0.378, 0.812) and discouragement in cases of off-target HbA1c levels (OR: 0.488; 95%CI: 0.337, 0.706) were associated to low adherence.

Conclusion: Adherence to self-monitoring blood glucose in patients with diabetes varies depending on age and on being recommended >21 measurements/week as a common factor associated with low adherence in all age groups. Other variables associated with poor adherence by age group are: alcohol intake in young patients, confidence in glycemic perception in middle age and lack of motivation for poor control in older people.

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The relation between illness perception, diabetes distress and depression in type 2 diabetes patients: pilot study in a Romanian sample

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Background and aims: Depression has a major negative effect on diabetes outcome. Illness perception and diabetes distress may contribute to emotion-

al and somatic depressive symptoms. Our aim was to investigate the relationship between illness perception, diabetes distress and depression.

Materials and methods: 204 type 2 diabetes patients were included in the study. Revised Illness Perception Questionnaire (IPQ-R) and Diabetes Distress Scale (DDS) were used to investigate illness burden. Beck Depression Inventory was used for depression. Linear regression analysis was used to evaluate the association between the five components of IPQ-R (illness identity, causes, timeline, consequences and cure-control), respectively four components of DDS (emotional burden, physician-related distress, regimen-related distress and diabetes-related interpersonal distress) and emotional and somatic symptoms of depression.

Results: Mean age of the participants was 64.61 years (range 32-81 years) and the mean HbA1c 7.43% (SD=1.46%). Depressive symptoms were found in 43.1% of patients of them 22.1% had subclinical depression. In depressive group, HbA1c was associated with somatic depressive symptoms ($r = .262$, $p < 0.05$). Emotional diabetes distress was identified as a mediator between illness perception identity and somatic depression: first and second mediation linear regression $R^2 = .174$, $p < .005$, $R^2 = .239$, $p < .005$, Sobel test z -score = 2.853, $p = 0.0043$. Emotional diabetes distress was also identified as a mediator between perception of illness consequences and somatic depression: first and second mediation linear regression $R^2 = .172$, $p < .005$, $R^2 = .217$, $p < .005$, Sobel test z -score = 3.170, $p = 0.0015$. Finally emotional diabetes distress was identified as a mediator between emotional representation of illness and somatic depression: first and second mediation linear regression $R^2 = .236$, $p < .005$, second mediation linear regression $R^2 = .216$, $p < .005$, Sobel test z -score = 3.1621, $p = 0.0015$.

Conclusion: Diabetes distress represents a mediator between illness perception and depression symptoms. Diabetes symptoms, perception of the illness severity and feelings of diabetes' complication inevitability having a significant effect on the mental representation of future life with diabetes. In return diabetes patients tend to be irritable, restless, with insomnia and fatigue. The results are important for clinical practice and may help these patients with cognitive restructuring concerning both illness perception and diabetes impact. A possible explanation for these findings may be the emotional and cognitive dysfunctional coping strategies in these patients.

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Depression influences on cardiometabolic profile responses to a lifestyle intervention in at-risk Brazilians

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Background and aims: Cardiometabolic diseases and depression are relevant public health problems that are commonly associated. Benefits of lifestyle interventions on improving cardiometabolic profile are well documented. Low adherence to a healthy lifestyle may be partially explained by the association with psychological disorders, such as depression. Aims: To evaluate whether depression modifies the impact of a lifestyle intervention on cardiometabolic profile responses in an at-risk sample of the Brazilian population.

Materials and methods: 129 individuals at risk of cardiometabolic diseases recruited from Brazilian public health care services (66% women, 56.2±11.6 yrs, BMI 30.3±5.4 kg/m²). Participants were allocated to one of two 18-month interventions on diet and physical activity. Socio-demographic and clinical data were obtained. Depressive symptoms were assessed by the Beck Depression Inventory and score ≥12 diagnosed depression. Changes in each of the five metabolic syndrome components (body adiposity: ≥5% decreased waist circumference and/or BMI; plasma glucose: ≥10% decreased fasting and/or post-load glycemia; blood pressure: ≥10% decreased mean blood pressure; HDL-cholesterol: ≥10% increased HDL; and triglycerides: ≥10% decreased triglycerides) were used to define responses to the intervention and improvement on cardiometabolic profile. Multiple logistic regression models were employed (by each gender) in order to analyze influence of depression in each of 6 cardiometabolic profile outcomes. All models were adjusted for age, physical activity and type of intervention. Inflammatory markers were added into the models as possible mediators of relationship between depression and cardiometabolic outcomes. Likelihood ratio test was employed to compare the results of a logistic regression model with interaction term and a logistic regression model without interaction term.

Results: Approximately 42% of individuals had depressive symptoms. Depressed participants had a higher BMI, cholesterol and diastolic blood pressure, and had a lower level of the quality of life and physical activity compared

to non-depressed individuals. Depressed women after 18 months of intervention were less likely to have an improvement in their plasma glucose (OR: 0.32; 95%CI: 0.12; 0.87) and blood pressure (OR: 0.29; 95%CI: 0.10; 0.94) compared to non-depressed women. Depression had no effect on the impact of intervention on body adiposity and lipid profile. Inclusion of inflammatory markers into the models did not influence the association of depression and improvement in cardiometabolic profile. Comparing models including or not the interaction variable (depression*interdisciplinary intervention) no difference in results was found.

Conclusion: Depression at baseline of a lifestyle intervention may predict a lower chance of improving long-term cardiometabolic profile particularly among women. This raises the necessity of increase health professionals awareness and public health policy makers' attention to depressed people at high cardiometabolic risk. We suggest a focus on screening and management of depression as part of the intervention programs for lifestyle changes in at-risk individuals.

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The impact of hyperglycaemia and obesity on hospitalisation costs and clinical outcome in general surgery patients

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Background and aims: The association between inpatient hyperglycemia and poor outcome in surgical patients is well established. It is not known if obesity increases rates of complications in surgical patients with and without hyperglycemia and diabetes.

Materials and methods: We analyzed the effects of obesity and hyperglycemia on hospitalization costs and clinical outcome in patients, with and without diabetes (DM), who underwent gastrointestinal, hepato-biliary and pancreatic surgery. Inpatient hyperglycemia was defined as BG ≥ 7.8 mmol/L. Overweight was defined by body mass index (BMI) between 25–30 kg/m² and obesity as a BMI >30 kg/m². Hospital cost was calculated using cost-charge ratios from Centers for Medicare & Medicaid Services. Hospital complications included a composite of major cardiovascular events, pneumonia, bacteremia, acute kidney injury, respiratory failure, and death.

Results: Among 2366 surgery patients, hyperglycemia was present in 1799 patients (76%) of whom 506 (28%) had history of DM and 1293 (72%) had no DM before surgery. Compared to patients with normoglycemia, those with DM and non-DM with hyperglycemia had higher number of hospital complications (5% vs. 21% vs. 20%, $p<0.0001$), longer hospital stay (5 days vs. 9 days vs. 9 days, $p<0.0001$), more readmissions within 30 days (10% vs. 18% vs. 17%, $p<0.0001$), and higher hospitalization costs (\$20,212 vs. \$78,690 vs. \$72,550, $p<0.0001$). Multivariate analysis adjusted for age, gender, BMI and type of surgery (laparotomy/open surgery) revealed that hyperglycemia in DM and non-DM patients was associated with a ~4-fold increased risk of complications compared to patients with normoglycemia [OR 4.4 (95% CI: 2.8,7.0) and 4.0 (2.6,6.1)]. In contrast, compared to normal-weight subjects (BMI 18.5–25 kg/m²), the presence of overweight status and obesity was not associated with increased risk of complications (17.3% vs. 15.7% vs. 16.1%, $p=0.77$), or hospitalization costs (\$58,313 vs. \$58,173 vs. \$66,644, $p=0.41$). Open surgery was associated with higher rates of complications (19.2% vs. 8.9%, $p<0.0001$) and higher hospitalization costs (\$67,191 vs. \$43,767, $p<0.0001$) compared to laparoscopic surgery; however, there were no differences in mortality, hospitalization costs or type of surgery among normal weight, overweight and obese (all, $p=NS$).

Conclusion: Perioperative hyperglycemia but not increasing BMI, in patients with and without diabetes undergoing gastrointestinal surgery, was associated with a higher number of complications and hospitalization costs.

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Seasonal fluctuations in nutrition variability in Europe: are patients with symptoms of a metabolic syndrome different?

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Background and aims: A lower nutrition variability seems to be related with an increased risk for metabolic and cardiovascular disorders. Seasonal changes in the availability of certain food items might influence food variability also in the high income countries of central Europe. We were interested in the possible dependency of nutrition variability over 4 seasons in an Austrian population especially with respect to differences between healthy people and patients with symptoms of a metabolic syndrome, including type 2 diabetes, hypertension and obesity (Body mass index, BMI, ≥ 30 kg/m²).

Materials and methods: For this purpose we have evaluated data from 3354 individuals (mean age 45.5 ± 18.9 years; mean BMI 24.3 ± 4.4 kg/m²) attending a medical outdoor center in an urban area between January 2006 and May 2013. Because of repeated controls during the evaluation period data from 4457 observations in healthy people and 352 in the 85 patients with symptoms of a metabolic syndrome were available. To obtain information on their "normal" nutrition behaviour, patients were requested to complete a semi-quantitative food frequency questionnaire including 51 different food items. Frequencies of consumption of food items were reported on a scale between 1 (seldom or never) and 6 (more than once daily). Based on this sample, we defined dietary diversity as the total number of different food items consumed during one week.

Results: Overall, nutrition variability was lower in patients with symptoms of a metabolic syndrome compared to the healthy control group. While nutrition variability revealed no significant seasonal dependency in the healthy group, low variability values were found in patients with symptoms of a metabolic syndrome during spring and fall.

Conclusion: The food variability seems to be lower in patients with a metabolic syndrome compared to a healthy control group. Regarding a possible seasonal influence the food variability revealed a decrease in the fall period. This lower food variability could be due to strict dietary recommendations, but also to rigid food behaviour, which becomes obvious when fresh local fruit and vegetables are available in autumn. Dietary counselling should include the recommendation for food variability.

PS 087 Individual perception of diabetes

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Somatic symptoms in Chinese patients with type 2 diabetes: frequency, risk factors, and the relation to quality of life

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Background and aims: Somatic symptoms are common in patients with chronic diseases which may reflect underlying emotional distress. We investigated the burden of somatic symptoms, risk factors and its associations with psychological health and quality of life in Chinese patients with type 2 diabetes.

Materials and methods: 2401 patients aged 18–75 years attending hospital-based clinics in 4 cities in China underwent detailed clinical-psychological assessment during a 12-month period in 2011–2012. Somatic symptom burden was measured using the 28-item Somatic Symptom Inventory (SSI); emotional distress was measured using the 21-item Depression, Anxiety Stress Scale (DASS); quality of life was measured using the Euroqol-5D.

Results: In this cross-sectional analysis, the mean (SD) age of the study cohort was 56.4 (10.6) years (46.6% women). Somatic symptoms were highly prevalent with a mean SSI score of 41.4 (15.1), and 14 of the 28 symptoms were reported by more than 30% of patients. Overall, 71.5% of patients reported at least one pain symptom, and 51.9% reported at least one gastrointestinal symptom. The most frequent reported symptom was trouble with vision (55.9%), followed by joint pain (44.9%), fatigue (44.8%), and muscles soreness (42.7%). In multivariate linear regression, after controlling for study sites, somatic symptom severity (adjusted $R^2=0.483$; $P<0.001$) was independently associated with women ($\beta=0.088$, $P<0.001$), long duration of diabetes ($\beta=0.049$, $P=0.008$), frequent hypoglycemia during the past 3 months ($\beta=0.075$, $P<0.001$), history of cardiovascular disease ($\beta=0.045$, $P=0.006$), sensory neuropathy ($\beta=0.088$, $P<0.001$), and emotional distress ($\beta=0.623$, $P<0.001$). Using stepwise linear regression, age ($\beta=-0.053$, $P=0.026$), women ($\beta=-0.07$, $P=0.001$), obesity ($\beta=-0.066$, $P=0.002$), frequent hypoglycemia in past 3 months ($\beta=-0.063$, $P=0.003$), sensory neuropathy ($\beta=-0.049$, $P=0.025$), and emotional distress ($\beta=-0.216$, $P<0.001$) were independently associated with poor quality of life. After inclusion of somatic symptom severity ($\beta=-0.257$, $P<0.001$), sensory neuropathy became non-significant ($\beta=-0.026$, $P=0.217$).

Conclusion: Somatic symptom burden is high in Chinese patients with type 2 diabetes which may reflect underlying emotional distress, suboptimal glycaemic control and sensory neuropathy.

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The Diabetes Self-Management Questionnaire (DSMQ) can detect inadequate self-care behaviour and help identify patients at risk of a negative diabetes prognosis

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Background and aims: Existing psychometric instruments to assess diabetes self-management often reveal weak or inconsistent associations with 'hard data' such as medical outcomes and HbA1c. To fill this gap, the Diabetes Self-Management Questionnaire (DSMQ) was developed, focussing on self-care

activities which directly impact medical diabetes outcomes. This study evaluates the questionnaire's practical utility in detecting high-risk patients at a tertiary diabetes centre.

Materials and methods: 226 people with diabetes (age 43 ± 15 y; 55% female; BMI 29 ± 7 ; 70% type 1 diabetes; illness duration 15 ± 10 y; 92% treated with insulin; HbA1c $8.9 \pm 1.6\%$) were assessed with the DSMQ and further questionnaires regarding diabetes acceptance (AADQ), coping with illness (FQCI), treatment satisfaction (DTSQ), diabetes distress (PAID), and depressive symptoms (CES-D); additional data (demographic variables, self-monitoring of blood glucose, HbA1c, and long-term complications) were gained from electronic patient records. People were then categorized by a median split of the DSMQ total score into groups performing 'adequate' ($n = 107$) versus 'inadequate' diabetes self-care ($n = 119$); the groups were compared regarding relevant outcomes using multivariate ANOVA (subsequently presented data are $M \pm SD$, F statistic, and effect size Cohen's d).

Results: After adjusting for sex, age, BMI, diabetes type, diabetes duration, and type of treatment, people performing 'inadequate self-care' compared to those with 'adequate self-care' showed stronger diabetes non-acceptance (31 ± 8 vs. 22 ± 6 , $F = 90.5$, $d = 1.24$), less active coping with diabetes (2.7 ± 0.9 vs. 3.4 ± 0.8 , $F = 18.1$, $d = 0.82$), lower diabetes treatment satisfaction (20 ± 7 vs. 24 ± 6 , $F = 21.3$, $d = 0.60$), higher diabetes distress (43 ± 21 vs. 33 ± 19 , $F = 17.0$, $d = 0.85$), and more depressive symptoms (24 ± 11 vs. 20 ± 11 , $F = 4.8$, $d = 0.36$). Moreover, they performed fewer blood glucose self-tests (3.3 ± 3.5 vs. 5.6 ± 2.4 times per day, $F = 24.7$, $d = 0.74$), consulted their diabetologist less often (1.9 ± 1.8 vs. 2.6 ± 2.3 times per half-year, $F = 6.6$, $d = 0.34$), had a higher HbA1c value (9.5 ± 1.5 vs. $8.2 \pm 1.4\%$, $F = 34.6$, $d = 0.87$), and showed a higher prevalence of retinopathy (28% vs. 14%, $F = 6.0$, $d = 0.35$).

Conclusion: The DSMQ yields excellent distinction between people with diabetes performing adequate versus insufficient diabetes self-care, thus enabling detection of people at high risk of a negative diabetes prognosis. The 16-item questionnaire is an efficient tool which may be used for screening and diagnostic purposes or clinical diabetes research.

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What drives patients? A cross-sectional survey of the effects and fear of hypoglycaemia on individuals, workplace, and patients' continued eligibility to drive

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Background and aims: The aim of this study was to investigate the effect of hypoglycaemia and fear of hypoglycaemia on the individual and society.

Materials and methods: An anonymous cross-sectional survey of Danish Diabetes Association members was conducted to investigate individual and societal consequences of hypoglycaemia.

Results: A total of 3,117 of 9,951 invited individuals with type 1 (32.2%) or type 2 diabetes (67.8%) completed the survey. The calculated incidence rates of self-reported severe hypoglycaemia was 2.9, 0.6 and 0.1 events per patient year (ppy) in patients with type 1 diabetes, Insulin using type 2 diabetes and non-insulin using type 2 diabetes, respectively; and incidence rates of self-reported mild hypoglycaemia were 99.0, 23.2 and 10.9 events ppy, respectively. Self-care strategies to avoid hypoglycaemia include maintaining higher blood glucose levels (45.7%) and reducing physical activity (15.7%). Few people take sick leave as a result of hypoglycaemia, but prolonged mental recovery ≥ 4 hours following an episode of mild or severe hypoglycaemia was reported by 8.7% and 31.0%, respectively. 9.3% of patients holding a valid driver license reported having 1 or more episodes of severe hypoglycaemia in the last year. Patients considering under-reporting of hypoglycaemia to maintain their driver license were more likely to have experienced severe hypoglycaemia (Odds Ratio [OR] 3.03, 95% CI 2.42–3.79, $p<0.0001$).

Conclusion: A high proportion of Insulin treated patients experience hypoglycaemia resulting in fear of hypoglycaemia and changes in self-care behaviour that may compromise glycaemic control. Many patients with a history of severe hypoglycaemia consider under-reporting hypoglycaemic events through concern over retaining their driver license.

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Perception of type 1 diabetes management by adolescents, parents and healthcare professionals: divergence of opinions influences metabolic and psychological outcomesS. Schmit¹, J. Jacobs¹, J.-P. Bourguignon², M. Fichelle³, S. Jellmann⁴, O. Ziegler⁵, C. De Beaufort³;¹Centre for Health Studies, CRP-Sante, Luxembourg, ²Adolescent Health Centre, CHU de Liège, Belgium, ³Department of Paediatric Endocrinology and Diabetology, Centre Hospitalier de Luxembourg, Luxembourg,⁴Clinical Infantile and Genetic Medicine, CHRU de Nancy - Hôpital d'Enfants, ⁵Department of Diabetology, Metabolic Diseases and Nutrition, CHRU de Nancy - Hôpital Brabois Adultes, France.

Background and aims: It is a major challenge for adolescents with type 1 diabetes to manage their disease, due to physical changes and psychosocial factors that characterise this period of transition. Although parents play an important role in supporting their children, responsibility is shifting. This may enhance conflicts between parents and adolescents, creating an important barrier to efficient metabolic control as well as to quality of life. Physicians and nurses represent another party supporting and influencing the youth's self-management. This study aims at comparing the perception of these different parties of several aspects of diabetes management and at identifying the influence of the convergence or divergence of these opinions on metabolic outcomes and the youth's well-being.

Materials and methods: Adolescents between 13 and 17 years visiting the outpatient clinic in 3 regions, as well as their parent(s), paediatric diabetologist and diabetes nurse filled out questionnaires containing questions on the adolescent's metabolic targets, treatment, well-being, quality of life, self-management and perception of self-management. Clinical data concerning blood glucose measurements, insulin regimen, episodes of severe hypoglycaemia and diabetic ketoacidosis and HbA1c level were collected via a Clinical Record Form. Answers of the 4 parties were compared to see whether convergent answers are related to metabolic control, well-being and quality of life. The study was approved by the three IRB's and Informed consent was obtained from all participants.

Results: The sum of distances between answers of the 4 parties on HbA1c targets and on well-being was correlated to HbA1c outcomes ($R = 0.448$, $p < 0.001$; $R = 0.232$, $p = 0.036$). The sum of distances between opinions on HbA1c aims, on hyperglycaemia management and on fear from hypoglycaemia were inversely correlated to the youth's self-management score ($R = -0.378$, $p < 0.001$; $R = -0.349$, $p = 0.001$; $R = -0.295$, $p = 0.003$).

Conclusion: These results confirm the importance of harmony between the different protagonists of the youth's type 1 diabetes management. The themes that seem to be most important are HbA1c targets, perception of the adolescent's well-being and perceptions of hyper- and hypoglycaemia management. Communication between protagonists proves to be extremely important in adolescent diabetes management, since the difficulty of this management may be related to the level of harmony between adolescents, parents and healthcare teams. These findings are an important element to be taken into account in the conception of patient education programmes.

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The impact of glycaemic variability on quality of life in adults with type 1 diabetesM. Reddy¹, R. Agha-Jaffar¹, P. Herrero², P. Georgiou², M. El-Sharkawy², P. Pesl², N. Jugnee¹, H. Thomson¹, C. Toumazou², D. Johnston¹, N. Oliver¹;¹Division of Diabetes, Endocrinology and Metabolism,²Centre for Bio-Inspired Technology, Institute of Biomedical Engineering, Imperial College London, UK.

Background and aims: We aimed to assess whether glycaemic variability (GV) has an impact on quality of life (QOL) in adults with established type 1 diabetes mellitus (T1DM) on either multiple daily injections (MDI) of insulin or continuous subcutaneous insulin infusion (CSII). There is evidence suggesting that GV reduces QOL in people with type 2 diabetes mellitus (T2DM), but this has not been fully explored in T1DM.

Materials and methods: Subjects wore a retrospective continuous glucose monitor (CGM) for 5-6 days and completed the diabetes quality of life questionnaire (DQOL). GV measures (SD, CONGA, LI, JINDEX, LBG, HBGI, GRADE, MODD, MAGE, ADRR, MVALUE, MAG) were calculated using the EasyGV version 9.0 software. A linear regression analysis was then per-

formed to assess whether there was a correlation between GV and measures of QOL.

Results: 57 adult subjects with T1DM (51% male, 65% on CSII, 35% on MDI, mean (SD) age 41 (13) years, duration of diabetes 21 (12) years, HbA1c 7.9 (1.1) %, body mass index 25.2 (4.0) kg/m²) were included in the analysis. No significant associations between GV measures and DQOL scores (Table 1) were demonstrated. The GV was significantly higher for those subjects on MDI compared to the CSII group (p -value < 0.05 for all GV measures), but no significant difference in QOL between the two treatment modality groups was observed.

Conclusion: Treatment with CSII is associated with lower GV compared to MDI. Interestingly, the outcomes from our study suggest that increased glycaemic variability does not impact overall quality of life in adults with T1DM, irrespective of whether they are on MDI or CSII.

GV measures	DQOL Total score All subjects (n=57)		DQOL Total score MDI subjects (n=20)		DQOL Total score CSII subjects (n=37)	
	R ²	p-value	R ²	p-value	R ²	p-value
SD	0.002	0.743	0.043	0.378	0.002	0.792
CONGA	0.004	0.641	0.032	0.453	0.0	0.991
LI	0.006	0.581	0.052	0.336	0.0	0.991
JINDEX	0.004	0.631	0.036	0.420	0.0	0.982
LBGI	0.005	0.617	0.050	0.344	0.018	0.434
HBGI	0.002	0.758	0.015	0.602	0.001	0.886
GRADE	0.0	0.882	0.002	0.852	0.001	0.843
MODD	0.009	0.479	0.046	0.362	0.01	0.570
MAGE	0.002	0.727	0.052	0.350	0.0	0.995
ADRR	0.01	0.463	0.143	0.100	0.0	0.961
M-Value	0.001	0.882	0.003	0.805	0.002	0.811
MAG	0.0	0.968	0.015	0.606	0.015	0.805

Table 1: Association between GV and QOL

Supported by: Wellcome Trust

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The relationship between negative emotions and quality of life in high risk patients with type 2 diabetes in Hong KongY. Junmei¹, R. Yeung^{1,2}, A. Luk^{1,3}, R. Wong¹, A. Kong^{1,3}, R. Ozaki^{1,3}, R.C.W. Ma^{1,3}, W. So^{1,3}, J.C.N. Chan^{1,2};¹Department of Medicine and Therapeutics, Chinese University of Hong Kong, ²Asia Diabetes Foundation, Prince of Wales Hospital,³Diabetes and Endocrine Centre, Prince of Wales Hospital, Hong Kong.

Background and aims: To measure the rate of negative emotions among Chinese type 2 diabetic patients with high cardio-metabolic risk, and the relationship between negative emotions and quality of life in Chinese patients with type 2 diabetes in Hong Kong.

Materials and methods: A consecutive cohort of patients with type 2 diabetes, aged 18-75 years, referred to our hospital for comprehensive diabetes complication screening with high cardio-metabolic risk from 4 March to 30 December 2013 were recruited. High cardio-metabolic risk was defined as poor glycemic control (HbA1c $\geq 8\%$), obesity (body mass index [BMI] ≥ 27.5 kg/m²), central obesity (waist circumference ≥ 90 cm in men or ≥ 80 cm in women), and/or chronic kidney disease (GFR < 60 ml/min/1.73m²). Clinical characteristics, self-care behaviors, and psychological measures were documented. Specifically, three types of negative emotions (depression, anxiety, and stress) were measured by the 21-item Depression, Anxiety, and Stress Scale (DASS-21). Quality of life was assessed by the 5-item Euroqol for health related quality of life (EQ-5D) and the Brief version of World Health Organization Quality of Life (WHOQOL-BREF).

Results: Among 370 eligible patients, 303 (82%) completed the assessments and 10 patients were excluded because they failed to complete $> 20\%$ of the

WHOQOL-BREF. Among the remaining 293 patients (age [mean±SD]: 59.1±9.6 years, 52.2% male), 67% of patients had at least one negative emotion, and 22% had significant levels of all three negative emotions. 50% of patients had moderate/ severe depressive symptoms, 43% had moderate/ severe anxiety symptoms, and 22% had moderate/severe levels of stress. Compared with patients with no/mild depression, patients who had moderate/ severe depression were younger (57.7±9.4 vs 60.3±9.7 years, $p=0.024$), had shorter diabetes duration (10.8±7.6 vs 12.8±8.2 years, $p=0.030$), used less insulin (38.8% vs 50.3, $p=0.049$) and exercised less frequently (43.3% vs 56%, $p=0.030$). They also had worse health related quality of life (EQ-5D: median[IQR] 0.85[0.75,1] vs 1[0.8,1], $p=0.004$), worse quality of life in all 4 domains of the WHOQOL-BREF: physical health (13.0±2.2 vs 15.3±2.1, $p<0.001$), psychological health (12.7±2.5 vs 14.6±2.5, $p<0.001$), social relationships (13.2±2.7 vs 14.7±2.5, $p<0.001$), and environment (12.9±2.8 vs 14.2±2.5, $p<0.001$). Similar results were found after stratifying patients by having anxiety or stress.

Conclusion: Patients with high cardio-metabolic risk were more likely to have significant levels of depressive and anxiety symptoms, and associated with worse health and non-health related quality of life.

Supported by: Merck Sharpe & Dohme

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Evaluation of cognitive functions and daily living activities of elderly diabetic patients under intensive insulin therapy

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Background and aims: Elderly population is a sensitive group. Insulin therapy needs strict compliance because of severe complications due to treatment. Cognitive functions and functionality may have an effect on the outcomes in this group. Our aim was to evaluate the cognitive functions and daily living activities of elderly diabetic patients who are under the basal-bolus intensive insulin therapy regimen. Our aim was to evaluate the cognitive functions and daily living activities of elderly diabetic patients who are under the basal-bolus intensive insulin therapy regimen.

Materials and methods: Our single centre, cross sectional study included 108 patients admitted to our Family Medicine outpatient clinic between February–June 2013, who are over 65 years of age and under intensive insulin therapy. Patients with established cognitive disorders, severe micro and macrovascular complications associated with diabetes mellitus were excluded from the study. Turkish validated versions of Mini Mental State Examination (MMSE) for cognitive functions, activities of daily living (ADL) and instrumental activities of daily living (IADL) scales were used to evaluate cognitive functions and daily living activities. MMSE score of ≤ 22 was considered as impaired cognitive functions. IADL scores vary between 0 and 17, higher score means patients are less dependent on somebody. ADL scores vary between 0–27, higher score means patients are more dependent on somebody. Fasting glucose and HbA1c levels were measured and history of mild and severe hypoglycemia were recorded. For group comparisons chi square and Student's T-tests were performed, $p<0.05$ was regarded as statistically significant.

Results: MMSE revealed 24 patients (22.2%) with impairment of cognitive functions. Fasting plasma glucose levels were 184.96±76.92 and main HbA1c levels were 9.06±2.02% in patients with impaired cognitive functions and fasting plasma glucose levels were 177.98±58.0 and main HbA1c levels were 8.64±1.33% in patients with normal cognitive functions ($p>0.05$). Number of severe hypoglycemic episodes was significantly higher in patients with impaired cognitive functions. 26.9% of patients with impaired cognitive functions reported at least one episode of severe hypoglycemia in the last 3 months whereas only 6.1% of patients with normal cognitive functions reported severe hypoglycemia. IADL and ADL scores of patients with impaired cognitive functions and normal cognitive functions were statistically significant. (IADL scores 11.04±4.89 vs 15.30±2.53 and ADL scores 8.96±3.26 vs 2.85±0.8, $p: 0.000$). Impaired cognitive functions were in relation with more hypoglycemic episodes and lower functionality.

Conclusion: Due to complexity of intensive insulin therapy regimen, cognitive functions and functionality of elderly diabetics should be carefully examined before deciding on intensive insulin therapy. In addition to that elderly diabetics under intensive insulin therapy should be evaluated closely by the physician.

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Stress: an important comorbidity of the metabolic syndrome

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Background and aims: It has become increasingly evident that psychological status affects all medical conditions, hence researchers have focused on these psychological status' roles especially on non communicable disease with almost unknown origin. The relationship of psychological distress with chronic metabolic disease such as diabetes mellitus, insulin resistance and dyslipidemia has been established. There are some evidences that have shown relations of lower physical well being and social function as indicators of Quality of life to Metabolic Syndrome. With this background, the present study was undertaken to investigate association of depression, anxiety, psychological distress and quality of life with metabolic syndrome in a sample of Indian population as a population with high prevalence of Metabolic Syndrome. Present study was undertaken with the aims of assessing levels of stress (Anxiety / Depressive symptoms) and Quality of Life in patients of Metabolic Syndrome and comparing it with healthy controls.

Materials and methods: A hospital based cross sectional case-control study was conducted in 200 patients attending medicine clinics and wards. They were analyzed separately as cases and controls based on the International Diabetes Federation Criteria for Metabolic Syndrome. Subjects who were a known case of any Psychiatric illness, having history of any major Psychosocial stressor in the past three months, suffering from any major medical or surgical illness or those patients who were on medications affecting emotion and mood were excluded from the study. Levels of stress were assessed using: General Health Questionnaire - 12 items, Hospital Anxiety and Depression Scale, Hamilton Rating Scale for Depression and Hamilton Rating Scale for Anxiety. Quality of life was assessed using World Health Organization Quality of life - BREF Version. Levels of stress and Quality of life in cases were subsequently compared with age, sex and social status matched healthy controls.

Results: A significant proportion of cases of metabolic syndrome reported psychological distress as evaluated by General Health Questionnaire. A significant proportion of cases of metabolic syndrome suffer from anxiety and depressive disorder as evaluated by Hospital Anxiety Depression Scale. Severity of anxiety as evaluated by Hamilton Anxiety Rating Scale is not associated with metabolic syndrome. Severity of depression as evaluated by Hamilton Depression Rating Scale is associated with metabolic syndrome. Cases of metabolic syndrome reported lower Quality of life scores thereby signifying poor quality of life in all the four domains as compared to controls, viz., Domain 1 (Physical health), Domain 2 (Psychological), Domain 3 (Social relationships) and Domain 4 (Environment) but the difference was statistically insignificant for the Domain 1.

Conclusion: This study proves the higher prevalence of depression as compared to anxiety and impaired quality of life in cases of metabolic syndrome. Although it suggest that psychological stress may have a causal role in etiology of metabolic syndrome yet there is a hen and egg dilemma that which comes first psychological stress or metabolic syndrome pointing out need for further studies to examine the effect of stress reduction techniques in reducing insulin resistance and improving the metabolic syndrome as a whole.

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Diabetes and work: a national register based cohort study on the potential influence of socioeconomic group, age, gender and ethnicity

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Background and aims: In countries with ageing populations it is anticipated that people will have to remain active in the labour market for longer and thus retire later in life. It is important, therefore, to address potential problems that might accrue from this significant social change. A number of studies have previously shown the impact of diabetes mellitus on, sickness absence, presenteeism and disability retirement among the workforce, yet the extent to which diabetes mellitus impacts on the working age population and the nature of its distribution within this group has not been comprehensively delineated. The aim of this study was to examine the impact and demographic distribution of incident diabetes mellitus on the working age population.

Materials and methods: The Danish National Diabetes Register was linked with socioeconomic and population registers covering the entire population. Twelve year diabetes incidence was analysed using multivariate Cox regression for 2,161,700 people aged 30–59 years at baseline.

Results: 93.2 % were of Danish origin, 3.6 % of non-Danish Western origin and 3.3 % of Non-western origin. 12 year incidence of diabetes was, respectively, 5.8%, 7.9 % and 11.4 %. Occupational activity was stratified by five main socioeconomic categories which are derived from the International Standard for the Classification of Occupations: professionals, managers, technicians, workers skilled at basic level and unskilled workers. Professionals were used as reference. The results of the survival analysis are shown in the table with 99 % confidence intervals.

Conclusion: Diabetes incidence increases with age, male gender, occupations with low socioeconomic status and among people of Non-western origin. The results indicate that undifferentiated upward adjustment of the retirement age has the potential to cause a marked increase in employees with diabetes, especially among already vulnerable groups. The social and economic implications are unknown and need to be subject of future research.

Hazard Ratios of 12 year diabetes incidence related to gender, age, socio-occupational group and country of origin among the total Danish population aged 30–59 year in 1999 (N=2,161,700)

	N	Diabetes (%)	RR	99% CI
Female	1,082,883	56,483 (5.22)	1.00	-
Male	1,078,817	73,530 (6.82)	1.35	(1.33 - 1.36)
30 - 39 years in 1999	787,415	21,127 (2.69)	1.00	-
40 - 49 years in 1999	697,339	39,873 (5.72)	2.25	(2.20 - 2.30)
50 - 59 years in 1999	679,946	69,013 (10.15)	4.12	(4.03 - 4.20)
Professionals	261,934	9,361 (3.57)	1.00	-
Technicians and associate professionals	333,512	13,369 (4.01)	1.27	(1.22 - 1.31)
Workers skilled at basic levels	785,490	42,813 (5.45)	1.65	(1.60 - 1.70)
Workers in elementary occupations	167,452	12,423 (7.42)	2.07	(2.00 - 2.14)
Non-employees	613,312	52,947 (8.49)	2.38	(2.31 - 2.45)
Danish origin	2,014,408	116,127 (5.76)	1.00	-
Western origin	77,228	5,941 (7.69)	1.31	(1.27 - 1.36)
Non-western origin	70,064	7,945 (11.34)	2.15	(2.08 - 2.21)

a relatively high risk of disability retirement. Targeting primary, secondary and tertiary prevention to the groups that need it most thus seems an essential strategy in attempts to prolong the working lives of individuals.

Socioeconomic Status	Diabetes	N	Cases	RR	95% CI
Legislators, senior officials and managers	No	55,823	926	0.92	(0.86 - 0.99)
	Yes	1,032	62	3.19	(2.49 - 4.10)
Professionals	No	286,401	4,724	1.00	-
	Yes	3,630	228	3.52	(3.08 - 4.02)
Technicians and associate professionals	No	377,340	8,486	1.43	(1.38 - 1.48)
	Yes	5,206	382	4.18	(3.77 - 4.64)
Workers in occupations that require skills at a basic level	No	1,005,542	36,040	2.69	(2.61 - 2.78)
	Yes	14,632	1,653	7.02	(6.63 - 7.42)
Workers in elementary occupations	No	225,856	14,086	4.58	(4.43 - 4.73)
	Yes	4,710	838	11.62	(10.80 - 12.51)
Rate ratios (RR) with 95 % confidence interval (CI) for disability retirement in Denmark 2001 - 2010 among employees with and without diabetes at baseline.					

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Relative rates of disability retirement among employees in Denmark with and without diabetes: a prospective analysis with 10 year follow-up

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Background and aims: Work and the workplace have been allocated a pivotal role in the future of health promotion, with economic and social stability predicated, in many accounts, on the assumption that more people will be fit, able and willing to work for longer. In this light, it is important to determine what socio-demographic trends might challenge this vision of future prosperity. Our ambition in this study was to estimate the proportion of disability retirements, among employees with diabetes, which could be attributed to socioeconomic status. This perspective was considered important since both incident diabetes and work-disability are marked by a social gradient. Obtaining more detailed and nuanced information on the relationship between diabetes and disability retirement is, therefore, undertaken in order to inform efforts to reduce disability retirement among people with diabetes.

Materials and methods: Using four national population registries, including the Danish National Diabetes Registry, we identified all employees in Denmark aged 20–59 years old at baseline (January 2001). These individuals were stratified according to their presence/absence in the diabetes registry and their socioeconomic status, and subsequently followed in our national registries until 31 December 2010.

Results: 29,210 people with diabetes and 1,950,962 people without diabetes were included in the analysis. The follow-up yielded 3,163 cases of disability retirement among people with diabetes and 64,262 cases among people without diabetes. With Professionals without diabetes as reference, the results indicate heightened levels of risk according to both presence of diabetes and socioeconomic status. The compound risk of being a person with diabetes and working in an occupation identified as having low socioeconomic status is clearly revealed in the 11.62 rate ratio for people with diabetes in elementary occupations.

Conclusion: The results show liability for early retirement among people with diabetes is especially acute for employees in lower socio-economic groups. The relative risks revealed in our study are especially concerning for the fact that there is evidence to suggest levels of social inequality in prevalent T2DM are on the increase. If current trends continue, therefore, there will, both proportionally and numerically, be more individuals with diabetes with

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Early evaluation of the ANAIS (Alimentación Normal Con Ajuste De Insulina) programme, a Spanish version of DAFNE

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Background and aims: The DAFNE (Dose adjustment for normal eating) programme promotes a flexible diet and has led to a reduction in clinical admissions and improved quality of life (QoL) in type 1 diabetes (T1D). ANAIS is a translation and adaptation of DAFNE to Spanish cultural and dietary patterns. Our aim was to evaluate the programme, using both clinical and patient-centred variables.

Materials and methods: This randomized, controlled, parallel group trial included patients with >2 years of T1D, age >18 years and HbA1c 7–12%. Participants were randomized (2:1 ratio) to participate immediately in a 5-day outpatient course (immediate ANAIS) or to do so after one year (delayed ANAIS). The primary endpoint was glycaemic control (HbA1c), measured 3, 6 and 12 months after the programme. We also evaluated the number of hypoglycemic episodes, weight, insulin dose, diabetes-related QoL (EsDQOL), treatment satisfaction (DTSQ), fear of hypoglycemia (MH-15), anxiety (STAI), depression (BDI), diet flexibility (open question) and achievement of self-defined goals (scale from -2 (much less than expected) to +2 (much more than expected)). Comparisons were made (Student's t, Wilcoxon's test and chi-square).

Results: So far, 80 patients (48 immediate ANAIS) have been included, 45% men, aged 34.6 (SD 11.35) years, diabetes duration 12.5 [range 3–47] years, with a BMI: 24.9 (3.9) kg/m² and a daily insulin dose of 0.75 (0.24) UI/Kg. In the intervention group, there was a 0.2–0.3% non-significant reduction in HbA1c at 3, 6 and 12, less hypoglycaemia at 6 months (2 [0–16] vs 1 [0–8]/week, $p=0.045$), and a trend towards a reduction in long acting insulin dose at 12 months (31.08 UI (12.5) vs 28.92 UI (12.6), $p=0.08$). No changes in weight were seen. Quality of life improved at 3 (96.7+/-19.9 vs 82.4+/-22.4 points, $p<0.0005$), 6 (87.1+/-22.7; $p<0.0005$) and 12 months (87.1+/-19.2; $p=0.002$). Treatment satisfaction increased at 3 (25.9+/-5.7 vs 28.2 (3.2) points, $p=0.019$), 6 (30.8 (4.6), $p<0.0005$) and 12 months 30.4 (7.7) $p=0.024$). Reduction in the fear of hypoglycaemia at 3 months (27.8 (9.3) vs 24.7 (8.5) $p=0.045$). Reduction in anxiety-trait at 1 year. No changes in depression. On average, the patients achieved their primary goal somewhat more than expected (1 point [(-1)-+2]). Before ANAIS, 25.5% considered their diet to be restricted. At 3 months, no patient had that opinion. In the control group, there were no significant changes in HbA1c or hypoglycaemia, but a weight increase was observed (baseline BMI: 24.85 kg/m² (4.57), 25.27 (4.69) at 3 and 25.07 (4.05) at 6 months, $p<0.005$) and total insulin dose increased at 12 months (0.80 UI/kg (0.27) vs 0.87 (0.3), $p=0.039$). QoL and fear for hypoglycaemia improved, but there was no change in treatment satisfaction or depression. Initially 38.7% considered their diet to be restricted and at 6 months, 31.3% still thought so.

Conclusion: So far, after the inclusion of a half of the patients, ANAIS has led to positive results in hypoglycemic events, weight and insulin dose, as well as in several measures of patient satisfaction, anxiety and depression and self-defined goal achievement.

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A new metric for objective evaluation of daily glucose profiles to obtain personalised recommendations for an individually sufficient diabetes care

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Background and aims: Structured glucose monitoring (CGM or SMBG) is an indispensable precondition for sufficient diabetes care and management. However, an objective and practicable tool for objective evaluation of measured glucose profiles to draw helpful, therapeutically relevant conclusions

from the measured glucose profiles is missing. It was the aim of our study to develop and to verify an objective evaluation tool for measured glucose profiles that might be able to bridge this existing gap in diabetes care and management.

Materials and methods: 1495 registered glucose profiles provided the database for this study. First, a factor analysis was performed to identify factors with major impact on the measured profiles. For each factor one parameter was selected and used for the development of a metric which can be used for automated, i.e. objective evaluation of the measured glucose profiles on the one hand and which provides recommendations for an improved diabetes management on the other.

Results: This study resulted in a Q-Score, which can be used for objective evaluation of glucose profiles, for automated identifying of potentials of improvement, and for providing recommendations for applying these potentials in practical diabetes care by predicting the expected outcome. To verify the Q-Score two diabetes specialists (DS) diagnosed independently 729 glucose profiles. The results were analysed for the inter-individual variation. There was a high correlation between the Q-Score and the results of both DS. The Q-Score was then tested for categorisation of glucose profiles. 729 profiles were categorised by one DS in very good, good, satisfactory, borderline, and not satisfactory and compared with the result obtained from the evaluation by applying the Q-Score. The Q-Score was significantly correlated with the results obtained from the evaluation of the DS.

Conclusion: The Q-Score combines all essential quality parameters to describe glucose profiles in only one parameter. The Q-Score is independent of subjective opinions and can be used therefore for automated evaluation of glucose profiles and to draw conclusions to generate patient related helpful recommendations for improving personalized diabetes care and management. The Q-Score has the high potential to become a practical tool in diabetes diagnosis and therapy.

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An innovative use of the ambulatory glucose profile to reveal the glycaemic effect of food

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Background and aims: The Ambulatory Glucose Profile (AGP) has been proposed as a standard reporting system for continuous glucose monitoring (CGM). The AGP reveals the progression from pre- to post-prandial periods, which may aid in refining nutrition & insulin therapy. Our aim was to explore the use of AGP to determine pre- and post-prandial identifiers and corresponding interventions.

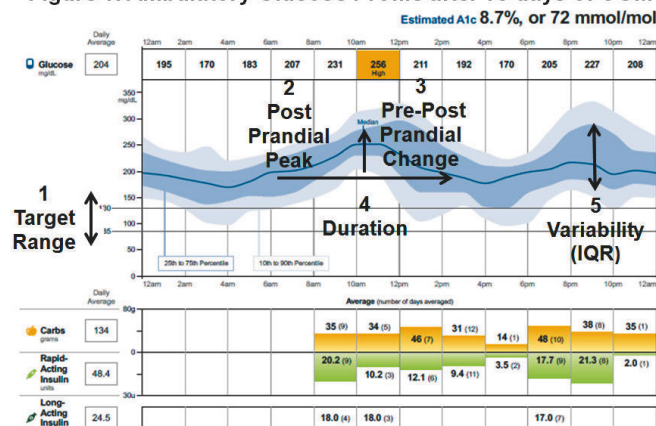
Materials and methods: AGP reports were evaluated from an intervention group of 53 subjects in a prospective, 9-site, 100-day study in the UK. Subjects were 18–82 years old, with type 1 or 2 diabetes, had HbA1c of 7.5–12.0%, and were managed by multiple daily injections of insulin. CGM (alarms turned off) was used by the intervention group. AGP reports similar to Figure 1 were evaluated using >14 days of CGM data.

Results: Five key metrics were identified to aid in prandial assessment and decision making (Figure 1). 1) Target Range identifies whether the pre-prandial and post-prandial median glucose levels are within target. If pre-prandial glucose is outside of target, consider assessing basal insulin and pre-prandial events or snacks; whereas, if the post-prandial glucose is outside target, consider assessing carbohydrate (carb) portions, insulin to carb ratio, and insulin timing. 2) Post-Prandial Peak is the highest point on the median curve typically occurring 1–3 hours after the start of the meal. Peak times may differ depending on meal composition. Peak time can aid in planning the timing of prandial insulin. Ideally the insulin action peak should match the post-prandial peak. 3) Prandial Glucose Change (pre-prandial - peak = change), reflects the balance of prandial insulin dose and carb. For excessive change, carbs may need to be decreased or the prandial insulin increased. 4) Duration of the Post-Prandial Curve is the amount of time after a meal to reach pre-prandial glucose levels. Longer durations can indicate high fat or high protein meals, gastroparesis, or inadequate prandial insulin coverage. The amount and timing of prandial insulin can be adjusted to lessen the duration of post-prandial rise. If post-prandial glucose does not return to baseline within the typical 3–5 hours and the next pre-prandial glucose starts at a higher level, an adjustment of basal and bolus insulin doses may be required. 5) Mealtime Glucose Variability is represented as the difference between the 25th–75th percentile curves or the interquartile range (IQR) after a meal. If carb type or amount varies on different days, the AGP may show greater variability or a wider IQR. Because AGP combines glucose profiles from multiple days,

evaluate the sources of variability such as food–insulin balance and the timing of meals across days. Decreasing variability or IQR may reduce the risk of hypo or hyperglycemia.

Conclusion: Analysis of AGP prandial periods can reveal the glycemic effect of food and insulin. AGPs can refine nutrition and insulin therapy relative to type and amount of carb, amount of dietary fat and protein, timing and amount of prandial insulin, and meal times.

Figure 1. Ambulatory Glucose Profile after 15 days of CGM



Clinical Trial Registration Number: NCT01713348

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Perioperative diabetes regulation and Protocol Compliance: the PC study
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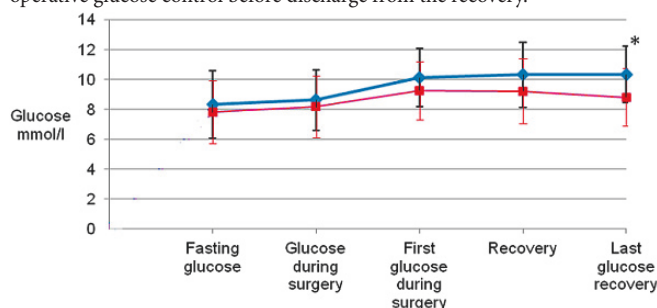
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Background and aims: Peri-operative diabetes regulation differs between hospitals. Compliance with regard to peri-operative glucose regulation protocols is notoriously low and evidence with regard to efficacy is lacking. There are, however, convincing studies demonstrating that improving post-operative diabetes control decreases complications. We therefore investigated whether implementing a modified and stricter peri-operative diabetes protocol improved compliance and peri-operative glucose regulation.

Materials and methods: We performed a prospective observational cohort study from March 2013 through July 2013, comparing compliance and glucose regulation following protocol A (conventional protocol) with protocol B (strict protocol). Protocol A prescribed a Glucose-Insulin-Potassium infusion, with 8 IU of insulin. Protocol B prescribed a Glucose-Insulin infusion, with 1/8 IU of the normal daily insulin dose. A pre-defined treatment algorithm to treat hyperglycaemia, glucose > 10 mmol/l, was added. In May 2013, we switched from protocol A to protocol B. Analyses were performed with the Mann Whitney U test and multivariate regression analyses.

Results: In total, 192 patients were included in protocol A and 183 in protocol B, mean age was 64 years and 54% was female, 10.4% of patients (n=20) had diabetes type 1, 86.5% (n=166) type 2, 3.1% (n=6) other. There were no differences between the patient characteristics in protocol A and B. The median compliance (IQR) was 67% (57–83) for Protocol A and 71% (57–83) for Protocol B, $p=0.04$. Differences between peri-operative glucose values are shown in graph 1. Adjusted for age, gender, ASA status, DM type, emergency procedures, fasting glucose and compliance, protocol B (red line) improved the proportion of patients that reached the target of < 10 mmol/l (OR 0.36, 95% CI 0.19–0.70, $p<0.01$) before discharge to the ward.

Conclusion: Implementing a stricter peri-operative diabetes protocol only slightly improved compliance, but significantly and relevantly improved post-operative glucose control before discharge from the recovery.



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The burden of “serial non-adherence” in type 2 diabetic patients

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Background and aims: Although medication adherence is recognized as a key concern for the treatment of Type 2 diabetes patients, there has been limited assessment of the scope of “serial non-adherence,” i.e. non-adherence to current diabetes regimen following non-adherence to prior therapies (diabetes or non-diabetes related), its predictability, and impact on patient/healthcare burden. The aim of this research was to characterize the healthcare burden associated with non-adherence with second-line anti-diabetic medication and to identify its key predictors, including the role of serial non-adherence.

Materials and methods: A retrospective cohort study was conducted using the Truven Health MarketScan® US Commercial health insurance database for the period 2008–2012. Patients were identified as first-line metformin users by requiring no prior anti-diabetic drug history going back at least 12-months before the date of the first metformin prescription. Patients in this group who subsequently initiated second-line (post-metformin) therapy were identified and selected for study. Diabetic regimen adherence was measured using the proportion of days covered (PDC), calculated as the total number of non-overlapping days of supply of any antidiabetic medication one year following the initiation of second-line therapy divided by 365 days. Adherence for selected non-diabetic medications, including lipid-lowering drugs and anti-hypertensives, was measured in a similar way during the baseline period. Non-adherence was defined as PDC<80%. Logistic regression models were constructed to identify predictors of adherence to second-line anti-diabetic therapy.

Results: Among the 46,789 patients who initiated second-line anti-diabetic therapy with at least 12 months of follow-up, 58% were non-adherent to anti-diabetic therapy during the first year after initiating second-line therapy. Among these patients, non-adherence to first-line metformin was very common (80% of patients) as was non-adherence to earlier non-diabetic medication (53% of those with non-diabetic prescriptions), with serial non-adherence accounting for over a quarter of overall non-adherence. Regression analysis showed that serial non-adherence was associated with a 4.67 times greater risk of non-adherence to second-line therapy ($p<0.0001$). Non-adherent patients were significantly (20% and 53%) more likely than adherent patients to experience any and severe hypoglycemia events, respectively ($p<0.001$ for both). After accounting for other confounding factors, non-adherent patients had average annual medical costs \$2,432 higher than adherent patients, which included \$1,678 higher annual inpatient costs during the first year following initiation of second-line therapy ($p<0.0001$ for both).

Conclusion: This study showed that more than half (58%) of patients initiating second-line anti-diabetic therapy are not adherent to therapy, with serial non-adherence (i.e., non-adherence to earlier diabetic and non-diabetic therapy) associated with much greater risk of non-adherence (OR=4.67). Second-line non-adherence is associated with higher risk of hypoglycemia and with higher healthcare costs. Since prior non-adherence is observable and appears highly predictive of later non-adherence, intervention strategies that target serial non-adherence may be particularly effective in improving the care of these diabetes patients.

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Do type 2 diabetes patients achieve therapeutic goals with medication adherence?

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Background and aims: In developed countries, adherence to drug treatment in chronic diseases hovers around 50 %. Improving adherence is key to effectively address the treatment of people with diabetes. Objectives: To determine the percentage of medication adherence and its relationship to the achievement of A1c, blood pressure and lipids goals in people with T2DM. To assess which factors are associated with lower medication adherence.

Materials and methods: A study was conducted in 31 medical centers specialized in diabetes in Argentina (12/ 2010- 05/2011), with a clinical and laboratory evaluation. The Morisky-Green test was used to assess adherence and WHO -5 for well-being and depression. Were taken as therapeutic targets A1c < 7 % , blood pressure < 140/90 mmHg and LDLcholesterol < 100mg/dl. (ADA). Statistics : Chi2, multiple logistic regression.

Results: 1,491 people were included; mean age 64.1 ± 11.3 years, duration of DM2 : 9.8 ± 7.8 years, 46.7 % female, BMI 31.4 ± 6 kg/m² and A1C $7.2\% \pm 1.4$. The mean WHO -5 test was $63.4 \pm 22.1\%$ with 39.2 % having values suggestive of depression. Adherence criteria was observed in 55% of the People. Factors that correlated with lower adherence to drug treatment by multiple logistic regression were: age under 65 years (OR: 1.36 , CI 1.08 to 1.72, $p < 0.009$), symptoms of depression WHO - 5 (OR: 1.59 , CI :1.01 -2, 59, $p < 0.04$), lower WHO -5 well-being (OR 1.02, CI :1.02 -104, $p < 0.04$), sedentary lifestyle (OR : 1.38 , CI 1.07 to 1.73, $p < 0.01$), fewer diabetologist visits in a year (OR: 1.37, CI 1.07 to 1.72 , $p < 0.01$) and not having health insurance ($p < 0.008$). In those who met adherence criteria 39.4% ($p < 0.001$) achieved A1c < 7%, 69.4 % BP targets ($p < 0.048$) and LDL- c 47.9 % ($p < 0.001$); having 25.6% achieved the three goals, 39.8 % two and 9.52% none.

Conclusion: In this sample of individuals with type 2 diabetes we found the frequency of medication adherence being 55 % and that 25.6 % of them achieved the three standard goals significantly. Predictors of lower adherence were age, symptoms of depression, lower welfare, sedentary lifestyle, diabetologist number of visits / year and not having health insurance.

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Regular exercise improves metabolic control and reduces chronic complications in patients with type 2 diabetes

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Background and aims: To investigate the current situation of exercise in patients with type 2 diabetes and the effect of different levels of exercise on metabolic control and chronic complications.

Materials and methods: A total of 5961 patients with type 2 diabetes from 50 centers in China were included in this nationwide, multicenter, cross-sectional survey. Patients were divided into three groups according their compliance to exercise: full, partial and poor compliance. Data were analyzed using analysis of variance and Chi-square methods.

Results: According to the questionnaire feedback, the patients (percentages) of full, partial and poor compliance groups were 2096(35.17%), 2398(40.23%) and 1466 (24.60%), respectively. There were significant differences in glucose and lipid level among three groups. Fasting blood glucose (7.70 ± 2.83 vs 8.17 ± 2.91 vs 8.55 ± 3.47 , $P < 0.05$), 2h postprandial blood glucose (10.80 ± 4.23 vs 11.71 ± 4.38 vs 12.16 ± 4.75 , $P < 0.05$), HbA1c (7.96 ± 2.12 vs 8.31 ± 2.23 vs 8.70 ± 2.32 , $P < 0.05$), total cholesterol (4.75 ± 1.33 vs 4.90 ± 1.51 vs 4.82 ± 1.36 , $P < 0.05$), triglycerides (1.90 ± 1.60 vs 2.09 ± 1.72 vs 2.25 ± 2.00 , $P < 0.05$), body mass index (24.12 ± 3.73 vs 24.61 ± 4.04 vs 24.80 ± 4.68 , $P < 0.05$), systolic blood pressure (129.26 ± 16.32 vs 129.97 ± 16.71 vs 131.86 ± 17.80 , $P < 0.05$), diastolic blood pressure (78.03 ± 9.89 vs 79.24 ± 10.38 vs 79.83 ± 10.81 , $P < 0.05$) of patients in the full compliance group were significantly

lower than those of partial and poor compliance group. The targeted rate of HbA1c (43.83% vs 27.98%, $\chi^2=59.444$, $P < 0.05$) in the full compliance group was higher than the poor compliance group. The morbidity of diabetic peripheral vascular disease (12.51% vs 15.96%, $\chi^2=8.580$, $P < 0.05$), diabetic nephropathy (12.56% vs 18.69%, $\chi^2=25.547$, $P < 0.05$), diabetic peripheral neuropathy (24.50% vs 29.33%, $\chi^2=10.715$, $P < 0.05$), diabetic retinopathy (28.84% vs 33.63%, $\chi^2=9.629$, $P < 0.05$) and diabetic foot (4.11% vs 9.14%, $\chi^2=47.724$, $P < 0.05$) of patients in the full compliance group were less than poor compliance group. For patients using insulin therapy, the incidence of hypoglycemia (45.25% vs 39.11%, $\chi^2=9.549$, $P < 0.05$) in the full compliance group was higher than the poor compliance group.

Conclusion: Regular exercise can improve glycemic, lipids, blood pressure, weight control and reduce morbidity of diabetic complications of patients with type 2 diabetes.

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Pedometer-based walking intervention and resting blood pressure in type 2 diabetes: a meta-analysis of randomised controlled studies

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Background and aims: Pedometer-based walking intervention, which is associated with improved physical activity and reduced body weight, has become popular among patients with type 2 diabetes (T2D). However, its effectiveness in resting blood pressure (systolic and diastolic) control has been poorly recorded. The aim of this meta-analysis of randomized controlled trials (RCTs) was to evaluate the association of pedometer-based walking intervention with resting blood pressure in T2D patients.

Materials and methods: PubMed, Web of Science and the Cochrane Library were searched from January 1994 to December 2013, using the following key words: pedometer*, accelerometer*, step counter, blood pressure, diabetes. RCTs in the English language were eligible for inclusion if they had utilized an intervention (lasting at least 8 weeks) using pedometer as a motivational tool to increase walking or walking speed in T2D patients, with more than 5 participants and reported changes in resting blood pressure. The summary estimates were made by a random-effects model.

Results: Of the 179 articles retrieved, 10 RCTs were included. Pedometer-based walking intervention was associated with a decreased resting systolic blood pressure by -1.13 mmHg (10 RCTs, 1020 participants; 95% confidence interval (CI): -2.79 to 0.52 mmHg), and a decreased resting diastolic blood pressure by -1.49 mmHg (9 RCTs, 928 participants; 95% confidence interval (CI): -4.04 to 1.05 mmHg) in T2D patients. However, both results were not statistically significant ($P = 0.18$ and $P = 0.25$, respectively). Univariate meta-regression analyses showed that neither intervention duration nor diary use was associated with a significant changes in resting systolic blood pressure ($P = 0.687$ and $P = 0.890$, respectively) or diastolic blood pressure ($P = 0.957$ and $P = 0.131$, respectively). No significant publication bias was detected by Begg's test or Egger's test in meta-analysis of resting systolic blood pressure ($P = 0.371$ and $P = 0.218$, respectively), or diastolic blood pressure ($P = 0.917$ and $P = 0.879$, respectively).

Conclusion: Current evidence regarding the effects of pedometer-based walking intervention in decreasing resting blood pressure remains insufficient in T2D patients. More well designed RCTs with guidelines for reporting data and details about the anti-hypertensive drugs use are needed.

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Does clinical inertia vary by personalised HbA_{1c} goal? A study of predictors and prevalence of clinical inertia in a US managed care setting

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Background and aims: Clinical inertia (CI) in type 2 diabetes mellitus (T2DM) care is defined as a failure to intensify treatment in patients with inadequate glycaemic control. There is a gap in understanding patterns of CI, particularly when considering the recent move towards personalized glycaemic targets rather than the standardized HbA_{1c} goal of < 7.0%. This study applied 3 different definitions of CI to a T2DM cohort from a large US managed care database to investigate the prevalence and predictors of CI. The 3 definitions of CI were based on different HbA_{1c} goals: (1) HbA_{1c} < 7.0%; (2) a modified target defined by Ismail-Beigi; and (3) Healthcare Effectiveness Data and Information Set (HEDIS) HbA_{1c} targets.

Materials and methods: Eligible adult T2DM patients were identified from the Optum Impact National Managed Care Database™ (IHCIS) between January 1, 2008–December 31, 2012. The index date was defined as the date of the first above target HbA_{1c} (the trigger HbA_{1c}) during the study period. Patients were required to have continuous health plan coverage for 6 months prior to index (baseline period) and for 6 months after index (response period). CI was defined as patients not receiving treatment intensification, defined as adding another oral antidiabetes drug (OAD), or initiating insulin or a glucagon-like peptide-1 receptor agonist within the response period. Predictors of CI were identified from the baseline period and evaluated by multiple logistic regression.

Results: The prevalence of CI was the highest for Definition 1, although the differences between the definitions were not substantial; 72.8%, 70.4%, and 70.6% for Definitions 1, 2, and 3, respectively. Common predictors of CI included higher age, higher baseline OAD usage, and a more recent index year, indicating a higher risk of CI over time (Table). Patients at a lower risk of CI included those with certain comorbidities, increased baseline non-diabetes medication use, an endocrinologist visit, and a higher index HbA_{1c} value. Differences between the predictors were also observed between the 3 definitions. Patients with baseline neuropathy, retinopathy, and a Charlson Comorbidity Index of 2–3 were less likely to experience CI according to Definition 1 only. Baseline myocardial infarction was identified as a predictor of CI for Definition 1 only.

Conclusion: These data suggest that the prevalence of CI in a US managed care setting was high and increased over the study period. The prevalence and predictors of CI were generally similar across all 3 study populations with different HbA_{1c} targets; however, there were some significant differences, particularly in the comorbidity-related predictors identified for Definition 1. This study provides some insights into clinical patterns of CI that could aid targeting of relevant T2DM subpopulations for improvement.

	Definition 1 HbA _{1c} > 7.0% (n = 79,805)	Definition 2 Ismail-Beigi Definition (n = 76,515)	Definition 3 HEDIS Definition (n = 79,070)
Predictors			
Age group at index date			
40–64 years	1.11 (1.04–1.18)**	1.12 (1.05–1.19)***	1.11 (1.04–1.18)***
≥ 65–74 years	1.30 (1.19–1.43)***	1.20 (1.09–1.32)***	1.20 (1.09–1.32)***
≥ 75 years	1.57 (1.37–1.80)***	1.35 (1.15–1.59)***	1.35 (1.15–1.58)***
Baseline Charlson Comorbidity Index (excluding diabetes)			
1	0.98 (0.93–1.03)	0.97 (0.92–1.02)	0.98 (0.93–1.03)
2–3	0.93 (0.87–0.99)*	0.95 (0.88–1.02)	0.94 (0.88–1.01)
≥ 4	0.82 (0.72–0.95)**	0.80 (0.69–0.94)**	0.82 (0.70–0.95)**
Comorbidities at baseline			
Neuropathy	0.90 (0.83–0.97)**	0.92 (0.85–1.00)	0.93 (0.86–1.00)
Retinopathy	0.91 (0.84–0.97)**	0.99 (0.91–1.07)	0.95 (0.89–1.02)
Myocardial infarction	1.19 (1.01–1.41)*	1.12 (0.92–1.35)	1.14 (0.95–1.40)
Mental illness	0.90 (0.84–0.96)***	0.91 (0.85–0.97)**	0.92 (0.87–0.98)*
Severe mental illness	0.89 (0.80–1.00)*	0.87 (0.78–0.97)*	0.87 (0.78–0.97)*
Number of OADs used at baseline			
2	1.78 (1.71–1.84)***	1.79 (1.72–1.85)***	1.79 (1.73–1.86)***
≥ 3	2.39 (2.27–2.52)***	2.41 (2.29–2.53)***	2.40 (2.28–2.51)***
Baseline HbA_{1c} (trigger HbA_{1c})			
≥ 8.0 to < 9.0%	0.44 (0.42–0.46)***	0.23 (0.21–0.26)***	0.45 (0.43–0.47)***
≥ 9.0%	0.26 (0.25–0.27)***	0.14 (0.12–0.15)***	0.27 (0.26–0.28)***
Resource use at baseline			
Endocrinologist visit	0.64 (0.60–0.68)***	0.62 (0.58–0.66)***	0.66 (0.59–0.67)***
Initiation year group			
2009	1.13 (1.08–1.18)***	1.13 (1.08–1.18)***	1.11 (1.07–1.16)***
2010	1.19 (1.14–1.25)***	1.16 (1.10–1.22)***	1.15 (1.10–1.21)***
2011	1.20 (1.14–1.27)***	1.17 (1.11–1.24)***	1.18 (1.12–1.24)***
2012	1.29 (1.19–1.39)***	1.21 (1.13–1.29)***	1.21 (1.13–1.30)***
Table. Predictors of CI During the 6-Month Response Period Based on 3 Target HbA_{1c} Definitions			
All data are odds ratio (Wald 95% confidence limits). Odds ratio indicates risk of clinical inertia; a ratio > 1 suggests an increased risk whereas a ratio < 1 suggests these patients were less likely to experience clinical inertia. Healthcare plan type, region, additional comorbidities, co-medications, hypoglycaemia, and hospitalization data were also included in the analysis, but are not shown in the table. Patient cohorts were not mutually exclusive. <p>*p < 0.05; **p < 0.01; and ***p < 0.001.</p>			

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Association of metabolic control and chronic complications with self-care behavior in Chinese type 2 diabetic patients: a nationwide, multi-centre, cross-sectional survey

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Background and aims: The purpose of this survey was to study the association of metabolic control and complications, with attitudes towards diabetes and self-care behavior in type 2 diabetic patients in China.

Materials and methods: Adults with type 2 diabetes from 50 medical centers across China covered 29 administrative divisions were eligible. The third version of the Diabetes Attitude Scale (DAS-3) and summary of Diabetes Self-Care Activities (SDSCA) were utilized to assess attitude towards diabetes and self-care behavior, respectively. Adequate metabolic control (hemoglobin A_{1c} < 7.0%, FPG < 7.2 mmol/L, 2hPPG 1.3 mmol/L in women, TC < 4.5 mmol/L, TG < 1.7 mmol/L) was defined as standards of medical care from the 2013 American Diabetes Association. The information of chronic complications was collected through primary interrogation and check.

Results: A total of 5961 respondents completed the questionnaires. As shown in table 1, patients with optimal glycemic control have higher scores of SDSCA in general diet (HbA_{1c}, P<0.001, FPG, P<0.001), exercise (HbA_{1c}, P<0.01), SMBG (HbA_{1c}, P<0.001), and medication adherence (HbA_{1c}, P<0.05, FPG, P<0.01, 2hPPG, P<0.001) than those with poor glycemic control. Patients with adequate lipid control have higher scores of SDSCA in general diet (TG, P<0.01), SMBG (TG, P<0.01, LDC, P<0.001, TC, P<0.05), and medication adherence (HDL, P<0.001, TC, P<0.05). People with targeted BMI (BMI ≤ 24 kg/m²) have higher scores of SDSCA in general diet (P<0.01), special diet (P<0.05), and but lower in medication adherence (P<0.001). However, there was no significant difference on scores of attitude towards diabetes between patients with optimal and poor metabolic control. On the other hand, the score of SDSCA in general diet (P<0.01), exercise (P<0.01), SMBG (P<0.01), and medication adherence (P<0.001) of patients without chronic complications including macroangiopathy, microangiopathy, neuropathy were significantly higher than those of patients with chronic complications. Meanwhile, showed less positive attitude on psychosocial impact of diabetes (P<0.05).

Conclusion: These data demonstrated that metabolic control and diabetic complications was directly associated with self-care behavior but not attitude. It suggests that the diabetes educators in China should pay more efforts on diabetes skill training towards self-care behavior for patients.

Table-1 Association of behavior with metabolic control and complications

		Scores of self-care behavior					
		General diet	Specific diet	Exercise	SMBG	Foot care	Medication adherence
total patients (n=5961)		4.9±2.2	4.2±1.5	4.1±2.5	3.0±2.7	4.4±1.9	6.0±2.3
Glycemic control variables							
HbA1c	<7.0%	5.3±1.9	4.6±1.5	4.6±2.5	3.4±2.7	4.7±1.9	6.1±2.2
	≥7.0%	4.8±2.2	4.3±1.5	4.1±2.6	2.9±2.6	4.4±1.9	5.9±2.2
	P value	0.000***	0.253	0.010*	0.000***	0.395	0.023*
FPG	<7.2 mmol/L	5.1±2.1	4.3±1.5	4.3±2.5	3.0±2.7	4.4±1.9	6.0±2.2
	≥7.2 mmol/L	4.6±2.3	4.1±1.5	3.9±2.6	2.9±2.7	4.3±1.9	5.9±2.3
	P value	0.000***	0.275	0.715	0.256	0.952	0.002**
2hPPG	<10.0 mmol/L	5.0±2.2	4.3±1.5	4.3±2.5	3.1±2.7	4.4±1.9	6.0±2.2
	≥10.0 mmol/L	4.7±2.2	4.1±1.5	3.9±2.5	2.9±2.7	4.3±1.9	5.9±2.3
	P value	0.177	0.403	0.188	0.848	0.349	0.000***
Lipid control variables							
LDL	<2.6 mmol/L	5.1±2.1	4.4±1.5	4.2±2.6	3.4±2.8	4.6±1.9	6.0±2.2
	≥2.6 mmol/L	5.0±2.2	4.4±1.5	4.2±2.5	3.2±2.7	4.5±1.9	6.0±2.2
	P value	0.216	0.877	0.316	0.000***	0.115	0.605
HDL	>1.0 mmol/L in men						
	>1.3 mmol/L in women	5.0±2.1	4.4±1.5	4.3±2.5	3.3±2.7	4.5±1.9	6.1±2.1
	≤1.0 mmol/L in men						
	≤1.3 mmol/L in women	5.0±2.1	4.4±1.5	4.0±2.6	3.2±2.7	4.6±1.9	5.9±2.3
	P value	0.450	0.886	0.170	0.227	0.173	0.000***
	P value	0.450	0.886	0.170	0.227	0.173	0.000***
TC	<4.5 mmol/L	4.9±2.2	4.4±1.5	4.2±2.5	3.1±2.7	4.5±1.9	6.0±2.2
	≥4.5 mmol/L	4.8±2.2	4.2±1.5	4.1±2.5	2.9±2.7	4.3±1.9	5.9±2.3
	P value	0.375	0.959	0.633	0.045*	0.176	0.012*
TG	<1.7 mmol/L	5.2±2.1	4.4±1.5	4.4±2.6	3.3±2.8	4.6±1.9	6.1±2.1
	≥1.7 mmol/L	4.8±2.2	4.3±1.5	3.9±2.5	3.2±2.7	4.4±1.9	6.0±2.3
	P value	0.003**	0.634	0.456	0.001*	0.181	0.430
Weight control variables							
BMI	≤24kg/m2	5.0±2.1	4.3±1.5	4.1±2.5	2.9±2.7	4.4±1.9	5.8±2.4
	>24kg/m2	4.7±2.2	4.2±1.5	4.1±2.6	3.0±2.7	4.4±1.9	6.1±2.1
	P value	0.003**	0.017*	0.196	0.480	0.544	0.000***
complications							
chronic complications	yes(n=3441)	4.7±2.2	4.1±1.5	4.1±2.6	2.8±2.6	4.5±1.9	5.8±2.3
	no(n=2520)	5.0±2.2	4.3±1.5	4.2±2.5	3.1±2.7	4.2±1.9	6.0±2.2
	P value	0.010*	0.384	0.004**	0.004**	0.539	0.000***
Macro-angiopathy	yes(n=1916)	4.8±2.2	4.2±1.5	4.1±2.6	2.9±2.7	4.5±1.9	5.9±2.3
	no(n=4045)	5.0±2.2	4.4±1.5	4.2±2.5	3.2±2.7	4.3±1.9	6.1±2.2
	P value	0.189	0.872	0.020*	0.067	0.233	0.000***
Micro-angiopathy	yes(n=2207)	4.8±2.2	4.2±1.5	4.0±2.6	2.9±2.7	4.4±1.9	5.9±2.3
	no(n=3754)	5.0±2.1	4.3±1.5	4.2±2.5	3.2±2.7	4.3±1.9	6.1±2.2
	P value	0.002**	0.238	0.003**	0.300	0.388	0.000***
Neuropathy	yes(n=1597)	4.8±2.2	4.2±1.5	4.0±2.6	2.9±2.7	4.6±1.8	5.9±2.3
	no(n=4364)	5.0±2.1	4.5±1.5	4.2±2.5	3.2±2.7	4.3±1.9	6.1±2.2
	P value	0.019*	0.165	0.100	0.001**	0.110	0.001**
*P<0.05 **P<0.01 ***P<0.001							

*P<0.05 **P<0.01 ***P<0.001

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Identifying consistent inconsistency in network meta-analyses: an illustration in type 2 diabetes

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Background and aims: Network meta-analyses (NMA) combine direct and indirect evidence across a connected network of trial comparisons to provide estimates of comparative efficacy for multiple treatments. They have been embraced by reimbursement agencies and recommended by health technology assessment bodies. A key concern is the comparability of treatment effect estimates from different trials. Where there is both indirect and direct evidence for one or more comparisons ('loops' in the network) it is possible to evaluate empirically the 'consistency' of the network. Inconsistencies between direct and indirect estimates may be alleviated by adjusting for trial differences using regression analysis or by excluding studies. However, as the networks of trial evidence become more complex, and potentially include multiple 'loops' it becomes more difficult to interpret evidence regarding 'inconsistency'.

Materials and methods: A variety of alternative methods have been proposed to examine inconsistency including (i) node-splitting whereby the difference between the direct and indirect evidence is calculated (where possible) for each comparison in the network, (ii) comparisons of the NMA to an 'inconsistency' model where the effect estimates for each treatment com-

parison in the network is allowed to be independent, (iii) a method which looks at inconsistencies between alternative study designs, (iv) plotting residual deviance for individual trial arms within the NMA, and (v) plotting mixed predictive p-values against a uniform distribution. We compare the implementation and interpretation of these methods using a previously published NMA in type 2 diabetes. In this analysis HbA1c was compared across six treatments (two GLP-1s at different administrations, placebo, and insulin glargine) in a network of 22 studies with multiple 'loops'. As in the original study, a random effect model controlling for baseline HbA1c was performed.

Results: The methods agreed in showing the presence of inconsistency with the network. For example, the inconsistency model showed an improved fit (DIC -62.35) compared to the consistency model (DIC -60.25). The node splitting method identified two treatment arcs as being inconsistent, liraglutide 1.8mg vs placebo and liraglutide 1.8mg vs exenatide QW. However, the methods varied in their ability to provide an overall 'test' of inconsistency across the network and their ability to identify which parts of the network contain inconsistencies. We highlight that none of the methods alone can identify individual studies as being the cause of inconsistencies and argue that we need to consider the whole structure of the network and the characteristics of the studies (in terms of treatments, subjects, and design) within the network.

Conclusion: Meta-analysis and decision makers often seek similarity across the network. However, we argue that we may be more confident generalising from treatment effects estimated from a well-connected and heterogeneous network if the estimates within the network prove to be consistent.

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A patient centered approach on newly-arrived persons to a diabetes clinic

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Background and aims: It's recognized that person-centred therapeutic education and group education with active methodologies promote experiences sharing, conviviality and stimulates learning among participants. The clinic introduced two structured programs for self-management education (DSME) addressed to newly-arrived patients covering a wide range of Diabetes Care in an integrated way. It's a comprehensive program of patient-centred care designed to increase autonomy, promote better adherence to treatment, and thus better metabolic control. We aim to perceive the programme's practical feasibility and people's adherence.

Materials and methods: Programme 1: lasts for 04:30h; During this period the person performs several tests: blood samples, EKG and retinography. A nurse performs a foot screening with risk assessment and foot care education. Then the person participates in two group sessions: a session with nurse guidance addressing pathophysiology of diabetes, relating the various important aspects in treatment and self-control, as well as doubts clarification and a final session about healthy eating, its importance for the metabolic control and lipid profile, and role of exercise in controlling diabetes (guided by a dietitian/nutritionist). The average time between the program and the 1st medical consultation at the clinic is 4 weeks. Programme 2: lasts for 3 months, divided in three group education sessions before the diabetes individual medical consultation (med). Sessions are guided by a facilitator using an IDF approved education tool, which provides an interactive verbal and visual learning experience, allowing groups engagement in an open and meaningful debate about diabetes. Sessions are divided by themes: the 1st (S1) leads to a reflection on their role in disease's self-management, the 2nd (S2) covers general concepts for healthy eating, and the 3rd (S3) is a physical activity session with a gym teacher. Patients are selected based on their age (50-80 y) and HbA1c (<10%), they are invited to attend programme 1 or 2 according to their convenience.

Results: Programme 1: A sample of 300 people (February to September 2013) with 60.4±10.3 years of age, an initial mean HbA1c of 8.7±1.5% and BMI mean of 28.2±4.6 kg/m² were analysed. No consistent changes were observed in terms of BMI or HbA1c in this group between the session and the medical consultation. Programme 2: We analysed a sample of 231 people (same period of time), with 68.3±8.8 years of age and an initial mean HbA1c of 9.1±0.7% and BMI mean of 35.3±3.2 kg/m². The drop-out rate was 10.8% at session 2 and 82% at session 3. No consistent changes were observed in BMI between the various groups. In terms of HbA1c it was observed a tendency of decrease between S1 and medical consultation directly related to the number of sessions attended (ΔA1c - Session 1: -0.27%; Session 2: -0.59 %; Session3: -0.85 %)

Conclusion: It's well known that active methods are a fundamental tool in group training. Here, the consistent decrease in HbA1c (programme 2) achieved independently of weight loss, hints to the impact of sharing solutions among peers by boosting diabetes acceptance, well-being and development of autonomy with DSME. The longer duration of this program also enables a slower integration of knowledge and skills in the daily life. However, the high drop-out before the exercise session advises us to consider alterations on program implementation, further encouraging patients participation.

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Relation between quality of care indicators of diabetes and prediction of hospitalisation and mortality for heart failure

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Background and aims: Heart failure (HF) is a major complication of diabetes especially as a consequence of ischemic heart disease. With the aim of evaluating whether a panel of quality of care indicators, as suggested by current guidelines, are related to the risk of hospitalization or subsequent mortality for HF, this study has retrospectively followed up, along a period of eight years (from 2005 to 2012) a cohort of diabetic patients living in Tuscany, a region of centre of Italy.

Materials and methods: The database used for this investigation was obtained from linking three datasets: the first concerning all hospital discharges with main diagnosis of heart failure (ICD-9-CM 402 to 428) from all Tuscan hospitals over the period 2005 to 2012, the second was the general population registry of all inhabitants of Tuscany and the third a dataset containing the registry of all known diabetic patients from Tuscany. This latter gave information about whether patients did perform annual assessment of HbA1c, eye examination, serum lipids, creatinine and microalbuminuria. In addition, from this dataset it was possible to evaluate whether patients were given at least 2 annual drug prescription of ACE-inhibitors (ACEI), aspirin (ASA) or lipid-lowering drugs (LIPID).

Results: On a total of 95,205 diabetic patients (47,762 males and 47,443 females), followed up over eight years, we counted 4,494 hospitalizations for HF (2,131 in males and 2,363 in females). After using a Cox proportional hazard model, the hazard ratio (HR) of hospitalization for HF, adjusted for age and Charlson co-morbidity index was inversely related with the annual execution of HbA1c, microalbuminuria or of lipid profile both in men (HR:0.923;0.896-0.952(95%CI), 0.972;0.949-0.995 and 0.895;0.870-0.920), and in women (HR:0.908;0.883-0.934, 0.968;0.945-0.991 and 0.900;0.878-0.923). The annual evaluation of serum creatinine, was on the contrary a positive predictor of risk (HR:1.306;1.260-1.353 in males and 1.359;1.315-1.405 in females). Annual eye examination was associated with a decrease in the risk of hospitalization for HF only among females (HR:0.957;0.937-0.977 among women and HR:0.986;0.964-1.008 among men). Finally, the composite indicator including the prescription for at least five years of ASA, ACEI and LIPID was significantly related with an augmented risk of HF-related hospitalization in both males (HR:1.285;1.130-1.456) and females (HR: 1.192;1.028-1.375). After HF hospitalizations 997 all-cause deaths were recorded and, by using a Cox model adjusting for age, sex and Charlson index, the survival after HF-hospitalization was significantly predicted by a low Charlson index (HR:0.928;0.864-0.996) and, again, by a lower number of annual evaluations of serum creatinine (0.935;0.886-0.985). No other indicator was significantly correlated with the prediction of post-hospitalization fatal events

Conclusion: In this cohort of Tuscan diabetic patients, over a eight-year period the annual execution of HbA1c, microalbuminuria, lipid profile and eye examination were inversely related with the risk of hospitalization for HF. Prescription of ACEI, ASA and lipid-lowering drugs was, on the contrary, associated with a significant increase in predicted hospitalization risk. Finally, post-hospitalization mortality risk was predicted by a higher Charlson index and by a higher rate of annual serum creatinine evaluations.

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Quality of care in type 1 diabetes in Italy: focus on gender differences

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Background and aims: We evaluated the quality of care according to gender in type 1 diabetes (T1D) in 300 diabetes outpatient clinics participating to "The Annali AMD" initiative. The Italian Association of Diabetologists (AMD) identified a set of quality indicators used for benchmarking activities since 2006, including process and intermediate outcome measures, as well as indicators of treatment intensity/appropriateness. A quality of care summary score (Q score), based on a combination of process and outcome indicators (range 0-40), was also calculated.

Materials and methods: We report here clinical data of 28,802 T1D patients (54.5% men; 45.5% women), collected during 2011 and extracted from electronic medical records.

Results: Both men and women received similar evaluation for process indicators (HbA1c, Lipids, BP, microalbuminuria, foot and eye examination), whereas the outcome indicators and treatment intensity/appropriateness differed according to gender (Table). No gender differences as to age (F: 45±16 years; M: 45±17) and diabetes duration (F: 19±13 years; M: 18±13) were noted. Men were more often smokers than women (31.8% vs. 22.7%) and showed a higher BMI (25±4 vs. 24±4 kg/m²), but F showed higher prevalence of BMI ≥ 30 or ≥ 35. T1D women showed poorer metabolic control and more often did not receive lipid-lowering agents in spite of high LDL-cholesterol levels than men, while men had poorer blood pressure control, and higher percentage of micro/macroalbuminuria. However, the overall quality of care, as estimated by Q score, was similar in both genders. Regarding the therapeutic approach (MDI vs CSII), the percentage of women treated with CSII was higher than men (19.6% vs. 13.8%); women were slightly younger (42.2±16.9 vs 45.1±16.9) for the same duration of disease (18.3±12.5 vs 18.1±13.2). A greater proportion of subjects treated with CSII reached the HbA1c level below 7.0% but women experienced more difficulties in achieving the HbA1c goal even if treated with CSII (HbA1c < 7.0%: 25.1% F vs 31.2% M).

Conclusion: Despite a similar quality of care, T1D women still show a poorer metabolic control, with any type of treatment, and were less intensively treated for LDL-C, while men show a worse cardiovascular profile in terms of blood pressure, microalbuminuria, BMI and smoking habits.

Indicators of intermediate outcome	F (%)	M (%)	p
HbA1c ≤ 7.0% (≤ 53 mmol/mol)	20.4	25.6	<0.0001
LDL-C <100 mg/dl	41.5	41.4	0.91
BP ≤ 130/80 mmHg	69.5	61.5	<0.0001
Presence of micro/macroalbuminuria	24.7	30.2	<0.0001
Indicators of treatment appropriateness			
No lipid-lowering agents despite LDL-c ≥ 130 mg/dl	71.1	68.2	0.04
No antihypertensive treatments despite BP ≥ 140/90 mmHg	47.4	49.8	0.06
No ACE-I and/or ARBs despite micro/macroalbuminuria	8.9	8.7	0.58
Overall quality of care			
Q score <15	7.6	7.5	0.68
Q score >25	41.5	40.6	0.15

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A global study of unmet need for glycaemic control and predictor factors among patients with type 2 diabetes mellitus

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Background and aims: Information on the efficacy of various treatments for type 2 diabetes mellitus (T2DM) in achieving glycaemic control is available globally from a number of different sources. This study utilized data from randomized controlled trials (RCTs), clinical trial registries (CTRs) and electronic medical records (EMRs) to identify unmet need for improved prandial glycaemic control in patients with T2DM following initiation of basal insulin therapy in Europe (EU), Asia Pacific (APAC), the US, and Latin America (LATAM).

Materials and methods: Different levels of evidence were used as available for each country/region, including RCTs (EU, LATAM, and APAC), CTRs (CREDIT 4-y, 9 countries mainly EU; ALOHA 0.5-y, Japan; Asia-FINE, 11 Asian countries), and EMRs (Germany IMS-DA, UK THIN, and US GE). We evaluated hyperglycaemia status by categorizing as 'well controlled' (defined as endpoint fasting plasma glucose [FPG] at target [defined as FPG <130 or 140 mg/dL; cut-off depending on country-specific recommendations] and HbA_{1c} at target [defined as HbA_{1c} <7%]), 'residual hyperglycaemia' (FPG at target but not HbA_{1c}) or 'uncontrolled' (both FPG and HbA_{1c} above target). Logistic regression analysis was used to identify predictor factors from the RCT dataset.

Results: Analysis of RCT data showed that ~43–54% of patients with T2DM globally had residual hyperglycaemia (Table), and rates across CTRs (CREDIT 31%, Asia-FINE 34.9%, and ALOHA 36%), and EMR (26% [Germany], 36% [UK], and 25% [US]) datasets were similar. A comparison across the EU showed consistent residual hyperglycaemia between CTR and EMR databases. Significant predictor factors were identified from RCT data, including baseline HbA_{1c} (all countries/regions except Brazil), baseline FPG (UK and Japan), and duration of diabetes (Brazil).

Conclusion: The higher proportion of patients with residual hyperglycaemia in the RCTs may be due to the selected population in RCTs. However, irrespective of intrinsic source differences between the datasets, 25–54% of patients with T2DM globally had residual hyperglycaemia despite meeting FPG goals, indicating an unmet need for additional prandial glycaemic control.

Table: Glycaemic control in randomized clinical trials by region.

Region	N	Controlled ^a	Residual hyperglycaemia ^b	Uncontrolled ^c	Missing data
Europe	954	28.0%	54.4%	16.9%	0.7%
Latin America	417	26.9%	52.0%	20.9%	0.2%
Asia-Pacific	787	16.9%	42.7%	38.1%	2.3%

^aDefined as both HbA_{1c} and FPG at target; ^bDefined as HbA_{1c} above target despite FPG at target; ^cDefined as neither HbA_{1c} or FPG at target.

Supported by: Sanofi

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Metabolic outcome data from a type 2 diabetes clinic in Denmark between 2001–2012

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Background and aims: Multifactorial interventions offered patients with type 2 diabetes (T2D), lead to substantial reduction in morbidity and mortality. Subsequent the Steno-2 study, a clinic providing referred T2D patients with dysregulated diabetes, complications or poor compliance, was opened at a specialized Diabetes Center (DC). Patients undergo a six to nine month treatment programme consisting of lifestyle education and motivation, and individually tailored pluri-pharmacy targeting hyperglycaemia, hypertension,

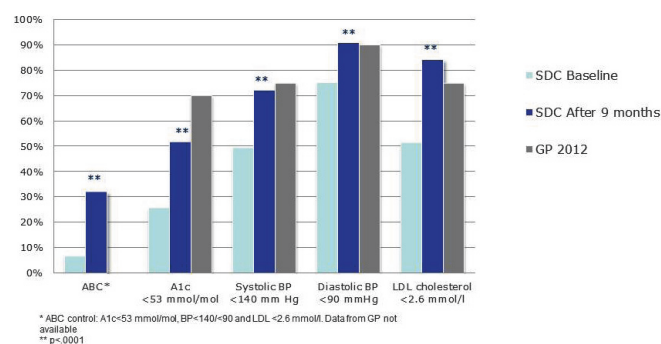
dyslipidaemia, and microalbuminuria. Our aim here was to find out the impact of this programme on short term metabolic outcomes and compare attainment of metabolic goals for patients in the general population to those of DC.

Materials and methods: Annual data from all patients with T2D referred to the type 2 clinic between Jan 1st 2001 and Jan 1st 2013 were extracted from the electronic medical records as part of quality of care assurance. In accordance with the national plan for diabetes care, the majority of patients (72%) were referred back to their general practitioner (GP) after being treated at the type 2 clinic. The remainder was retained at DC due to their level of disease complexity. Data up to nine months at type 2 clinic were followed. For comparison we used data from the Danish Adult Diabetes Database (DVDD) from 2012. Non-parametric statistic analysis was used throughout.

Results: We included a total of 4,143 patients (male 59%), with 489 (12%) patients going through more than one treatment programme. Mean age was 60 ± 12 years (mean ± SD) and mean duration of diabetes was 6.8 ± 6.6 years. DVDD contains data from 12,501 patients (male 56%) with T2D followed by GPs; mean age 68 ± 11 years, and mean duration of diabetes 6.5 years. The mean HbA_{1c} in patients with T2D followed by GPs was 48 mmol/mol (DCCT HbA_{1c} 6.5%), while that of referred patients was 67 ± 20 mmol/mol (8.3%) and decreased to 55 ± 14 mmol/mol (7.2%) by the end of programme (p75 mmol/mol); 32% at baseline and 9% after completion of the programme. The well-regulated group (HbA_{1c} <53 mmol/mol) increased from 25% to 52% of all after 9 months (p<0.0001). Mean body mass index (BMI) also decreased significantly, most notable from 2010; mean BMI from 31.3 ± 6.5 kg/m² to 30.9 ± 6.2 kg/m². This decrease was most prominent in those who were obese (BMI >30 kg/m²) at baseline, as well as in females (p<0.0001 for both). Mean systolic and diastolic BP levels decreased from 143 ± 21/82 ± 12 mmHg at baseline to 137 ± 19/80 ± 10 mmHg (p<0.0001). The percentage of patients below target pressure (<140/<90 mmHg) went from 45% at baseline to 70% after the programme (Figure). LDL-cholesterol decreased from 2.6 ± 1.0 mmol/L to 2.2 ± 0.9 mmol/L (p<0.0001); 52% of patients on target (LDL-cholesterol <2.6 mmol/L) at baseline and 84% after finishing the programme.

Conclusion: Our data show that the patients referred from GPs to a specialized DC are of appropriate complexity or dysregulated and that their metabolic control can be improved to near average GP level.

Metabolic Outcomes 2001–2012



PS 090 Individualised care

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Trends over 8 years in quality of care provided by Italian diabetes clinics to elderly patients with type 2 diabetes

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Background and aims: The prevalence of diabetes in people over 75 years approaches 30%, while at least 25% of all patients attending Italian outpatients diabetes clinics are older than 75 years. Clinicians who manage older people with diabetes require special skills to provide high-quality care. In the context of a continuous quality improvement initiative promoted in Italy by Associazione Medici Diabetologi (AMD Annals initiative), we evaluated the trends over 8 years in the quality of care provided by diabetes clinics to elderly patients (i.e. > 75 years).

Materials and methods: Overall, 300 diabetes clinics (about half of all clinics in Italy), all using electronic medical record systems, extracted data relative to the years 2004–2011. The proportion of patients with at least one value registered during each year (process measures), the percentage of patients reaching specific favorable or unfavorable targets (intermediate outcome measures), and rates of use of drugs were evaluated. In addition, a quality of care summary score (Q score) was calculated. The Q score, ranging between 0 and 40, is based on process and outcome indicators (HbA1c, blood pressure, LDL-cholesterol, microalbuminuria) and is closely related to long-term outcomes in diabetic patients.

Results: Over the years, there was an increase in the percentage of patients aged > 75 years (19.9% in 2004 vs. 27.2% in 2011) and in the prevalence of male patients (42% in 2004 vs. 46% in 2011). As compared to 2004 we observed after 8 years a slight increase in the mean age (79.9±3.8 vs. 80.5±4.1) and in the duration of diabetes (13.8±10.5 vs. 14.2±10.9). Table 1 shows quality of care indicators.

Conclusion: Care provided by diabetes clinics to elderly patients shows a significant improvement over the years. Elderly patients are more frequently monitored for blood pressure, lipid profile and microalbuminuria, show better intermediate outcomes and are less often treated with sulphonylureas. In addition we observe a better global quality of care with a significant increase in the Q score through the years.

	Indicator	2004	2005	2006	2007	2008	2009	2010	2011
PROCESS MEASURES	HbA1c	88.6	89.1	89.5	90.0	90.2	90.9	91.0	91.0
	Blood pressure	72.5	72.8	75.3	74.7	75.2	74.4	73.6	73.6
	Lipid profile	50.0	53.7	58.1	63.0	64.8	67.3	68.9	69.4
	Microalbuminuria	34.6	35.5	34.8	33.6	34.6	36.7	38.0	40.7
FAVORABLE OUTCOMES	HbA1c ≤ 7%	38.2	38.6	39.4	43.2	43.0	42.2	41.6	42.0
	BP < 150/90 mmHg	49.7	50.7	53.0	56.4	57.8	59.7	62.8	64.5
	LDL-C < 100 mg/dl	28.3	31.4	36.6	39.0	42.3	43.8	48.0	51.0
	HbA1c ≥ 9%	15.9	14.7	14.3	12.1	11.9	11.0	10.6	10.8
UNFAVORABLE OUTCOMES	BP ≥ 150/90 mmHg	50.3	49.3	47.0	43.6	42.2	40.3	37.2	35.5
	LDL-C ≥ 130 mg/dl	37.1	33.9	29.4	27.1	24.6	23.8	20.8	19.0
	Oral agents:								
	Metformin	32.8	32.8	35.8	38.1	39.8	41.0	42.4	43.6
TREATMENT	Sulphonylureas	40.2	38.5	38.7	39.2	38.1	36.5	35.3	34.0
	Insulin	22.9	23.7	25.2	28.1	30.6	31.8	33.3	34.1
	≥ 2 antihypertensive agents	22.4	26.6	31.0	35.9	39.5	41.7	44.2	44.7
	Lipid-lowering agents	12.8	17.2	22.4	28.2	32.4	36.6	40.3	42.3
GLOBAL	Q SCORE < 15	13.7	12.8	11.6	9.4	8.6	8.1	7.3	7.1
	Q SCORE ≥ 25	19.2	21.0	23.9	27.8	30.3	32.5	34.9	35.7

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Comparing the use of patient level data to an average patient profile within a type 2 diabetes simulation model

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Background and aims: To accommodate patient heterogeneity and complex treatment pathways, type 2 diabetes models employ simulation techniques beyond the basic Markov process. Yet averages are commonly relied upon

when defining patient populations and treatment effects; in turn clinicians may struggle to relate the results of such modelling to the full spectrum of patients they see in practice. This study investigates the value of fully modelling between patient variation by comparing patient and cohort level model inputs within a published simulation model, based on the UKPDS68 outcomes equations.

Materials and methods: Anonymised UK patient data was obtained from The Health Improvement Network (THIN) describing 2,251 patients initiating dual therapy. Simulations were initialised by applying either the average patient profile to the cohort, or by individually replicating each patient's profile, followed by the collation of model outputs over all replications. The impact of utilising patient level baseline and treatment effect data was compared to the average patient profile through evaluation of total costs, benefits and complication rates, predicted over a medium-term horizon of 20 years.

Results: On average patients were aged 63.36 (±11.14) at baseline, with the following risk factor profile; HbA1c 8.39 (±1.23) %, total cholesterol 4.18 (±0.92) mmol/L, systolic blood pressure 135.07 (±14.76) mmHg and weight 89.85 (±19.01) kg. The mean treatment effect on HbA1c was a reduction of 1.01 (±1.23) %. Over 20 years, fewer macrovascular and microvascular events (-82/1,000 patients) and higher all-cause mortality (+17/1,000 patients) were predicted when using patient level data inputs compared to the average patient profile. Differences in the simulated frequency and timing of deaths were driven primarily by variation in baseline age and led to fewer estimated life-years (-0.66), quality adjusted life-years (QALYs) (-0.59) and costs (-£551) per patient. Patients estimated to have both lower costs and higher QALYs than those associated with the average patient profile were younger, with higher HbA1c and cholesterol and lower blood pressure at baseline.

Conclusion: Modelling results differ depending on the use of patient level or average cohort model inputs. Patient level data may provide insight into the type of patients in whom therapy is likely to be most beneficial. Furthermore, it enables the accurate simulation of correlations between patient characteristics and treatment effect, which are rarely accounted for as part of a standard probabilistic sensitivity analysis.

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Is there an evidence base for the clinical features used to differentiate type 1 from type 2 diabetes? A systematic review of the literature

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Background and aims: Clinicians predominantly use clinical features to differentiate Type 1 from Type 2 diabetes but no guidelines quote an evidence base for which features are the most discriminatory. Whilst Type 1 patients are thinner, diagnosed younger, and rapidly go onto insulin, the best cut offs of these criteria are not provided. Misclassification of diabetes is widespread (7–15% of cases) and will result in patients receiving the wrong treatment. The key difference between Type 1 and Type 2, that determines treatment, is that patients with Type 1 diabetes develop absolute insulin deficiency. We aimed to systematically review the literature to identify which clinical criteria could be used to discriminate Type 1 and Type 2 diabetes, using insulin deficiency as a gold standard.

Materials and methods: The search strategy took the form of: (terms for diabetes) AND (terms for C-Peptide). 14 databases including MEDLINE and EMBASE were searched. All diagnostic accuracy studies, published since 1979, using clinical criteria to predict insulin deficiency (defined by C-peptide concentrations) were included. There was no restriction on race, age, language, or country of origin. Data synthesis was largely descriptive.

Results: 10,917 abstracts were screened, and 231 full texts reviewed. 10 references were identified for final inclusion. Studies varied by age, race, year, and proportion of participants who were C-peptide negative, prohibiting formal meta-analysis. Consistent predictors across studies were age at diagnosis (the most discriminatory feature; mean ranking of predictors by discriminatory ability within studies=1.4), insulin treatment/time to insulin (mean rank=1.7), and BMI (mean rank=2.6). Discriminatory cutoffs were age at diagnosis 30–40 years, time to insulin 1–2 years, and BMI 27–28 kg/m² (>66% sensitivity and specificity for all). BMI added little over age at diagnosis and/or time to insulin (<1% improvement in classification).

Conclusion: Studies of the accuracy of clinical criteria to define Type 1 diabetes are surprisingly few given the importance for treatment of patients. Despite finding only 10 studies, and considerable heterogeneity between studies, age at diagnosis (35 years) and time to insulin treatment (1.5 years) were consistently the most discriminatory. BMI, despite being widely used in clinical practice, adds little to these 2 criteria.

Supported by: NIHR

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Admission albumin levels are associated with increased risk for hypoglycaemia during hospitalisation as well as poor 1-year survival, among patients with diabetes mellitus

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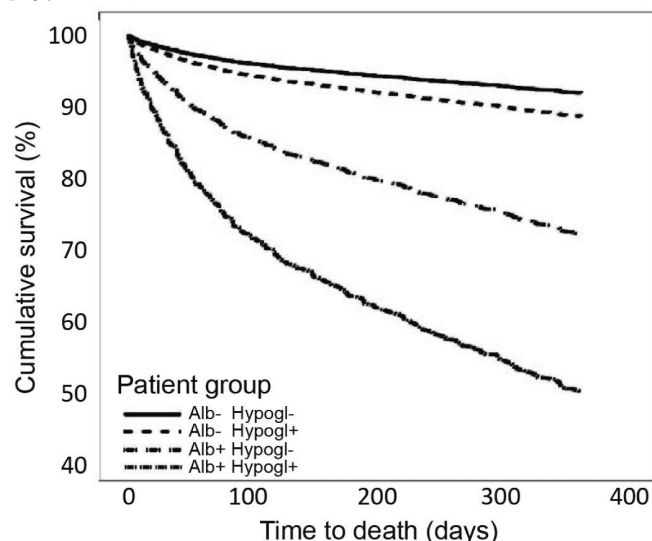
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Background and aims: Among non-critically ill patients, there are detrimental effects of hypoglycemia during hospital stay. Hospitalization-associated hypoglycemic events may occur in patients with diabetes mellitus that are prone to develop it. We studied the association between admission hypoalbuminemia and the risk of hospitalization-associated hypoglycemia, among patients admitted to internal medicine departments.

Materials and methods: In this retrospective analysis of electronic medical records, we included all 2599 patients with diabetes mellitus and documented admission albumin levels (Mean age 71.7±13.0 years, 48.4% males, 65.9% of all patients with diabetes), admitted to internal medicine departments during 2009. All glucose measurements were computerized using an institutional glucometer. Patients were categorized into 4 groups according to hospitalization-associated hypoglycemia (yes/no) and admission albumin levels below 3.5 g/dL (yes/no).

Results: Patients with hypo-albuminemia had higher rates of hypoglycemia and severe hypoglycemia during the admission despite similar HbA1c and average glucose control during the admission. Only creatinine (OR 1.237, 95% CI 1.098–1.392, p<0.001) and albumin levels (OR 0.417, 95% CI 0.342–0.509, p<0.001) affected the risk for hypoglycemia. In addition, Patients with hospitalization-associated hypoglycemia combined with admission hypoalbuminemia had the lowest survival rates (hospital and 1-year), compared to the other groups (OR 7.419, 95% CI 5.255–10.473 respectively, p<0.001). Patients with hypoglycemia but with normal albumin levels had a similar survival curve compared to diabetes patients without hypoglycemia nor hypoalbuminemia.

Conclusion: Low albumin levels upon admission are associated with increased risk for hypoglycemia during hospitalization. The occurrence of hypoglycemia among patients with low albumin levels is associated with reduced 1-year survival compared to both diabetes patients with hypoalbuminemic but without hypoglycemia, as well as diabetes patients without hypoglycemia and normal albumin levels.



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Real-world treatment switching patterns in patients (pts) with type 2 diabetes mellitus (T2DM) on basal insulin (INS)

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Background and aims: Increasing treatment options have empowered but also complicated disease management. We explored transitions from INS to other therapies in a real-world setting.

Materials and methods: We analysed data from Diabetes FORWARD, a practice-based research network focused on T2DM pts and their providers across the US (September 2012–December 2013). Pts aged ≥ 18 years, diagnosed with T2DM and with a start date for INS ≤ 12 months (mo) before enrolment were eligible. Based on prescription data from electronic medical records, pts were stratified by treatment switches at 3, 6, and 9 mo as those who: remain on INS; switch to oral antidiabetes drugs (OADs); switch to a glucagon-like peptide-1 receptor agonist (GLP-1) ± INS; or switch to other insulin (85–90% added prandial insulin to INS). As in any observational study, not all pts contributed data at each time point; therefore, 3, 6 and 9 mo groups were defined.

Results: 300 pts were eligible. At 3 mo, 81% of pts remained on INS, decreasing to 65% at 6 mo and 56% at 9 mo; proportions of pts switching to other therapies increased. At baseline (BL) there were no differences in age, gender, race/ethnicity, education level, or number of OADs between groups. Pts remaining on INS or switching to other insulin had more Medicare coverage. In the GLP-1 group, more pts had private health insurance, although numbers are small (Table). At all time points, pts transitioning to OADs reported worse general health than those remaining on INS; those transitioning to other insulin reported the worst general health (Table). There were no differences in BL HbA1c between groups, although BL HbA1c was numerically highest for pts switching to other insulin (Table). HbA1c decreased in all groups except for pts transitioning to other insulin which increased. Greatest reductions in HbA1c were seen in the OAD and GLP-1 groups (Table). Pts switching to GLP-1 or other insulin had higher BL weight; those in the 6 and 9 mo OAD groups had the lowest weight. Greatest weight loss was observed in the GLP-1 group (data not shown).

Conclusion: Patterns observed here suggest that, in the practice setting, pts with T2DM transitioning from INS to OADs report worse health, have a lower weight, and relatively good glycaemic control. However, those transitioning to GLP-1 report good health, higher weight, and better glycaemic control. Pts transitioning to other insulin report poorer health, higher weight, and the worst glycaemic control. Medicare beneficiaries had the least access to GLP-1 suggesting insurance influences provider decisions. Other reasons for switching therapy may be intolerance to, or ineffectiveness of, previous therapy and disease progression.

Characteristic	INS	Switch to OADs	Switch to GLP-1 ± INS	Switch to other insulin	p-Value
Private health insurance at BL, % (n/N)					
3 mo	38.5 (34/244)	35.0 (7/26)	40.0 (2/5)	23.1 (1/13)	0.7237
6 mo	30.9 (76/195)	39.9 (14/36)	45.5 (5/11)	25.0 (2/7)	0.2887
9 mo	40.8 (69/169)	44.4 (16/36)	50.0 (3/6)	16.0 (4/25)	0.0887
Medicare coverage at BL, % (n/N)					
3 mo	38.9 (35/244)	15.0 (3/26)	0 (0/5)	30.8 (4/13)	0.0536
6 mo	36.5 (79/195)	16.7 (6/36)	0 (0/11)	48.1 (12/27)	0.0056
9 mo	39.1 (66/169)	19.4 (7/36)	0 (0/6)	52.0 (12/25)	0.0118
General health at BL, % (n/N)					
3 mo					0.0269
Excellent/very good	1.7 (4/229)	5.3 (1/19)	0 (0/5)	0 (0/2)	
Very good	16.2 (37/229)	5.3 (1/19)	40.0 (2/5)	0 (0/2)	
Good	40.8 (93/229)	42.1 (8/19)	40.0 (2/5)	25.0 (1/2)	
Fair	32.3 (74/229)	23.1 (4/19)	20.0 (1/5)	50.0 (2/2)	
Poor	9.2 (21/229)	26.3 (5/19)	0 (0/5)	25.0 (1/2)	
6 mo					0.5434
Excellent/very good	1.6 (3/183)	6.1 (2/13)	0 (0/8)	0 (0/6)	
Very good	16.4 (30/183)	9.1 (4/13)	25.0 (2/8)	7.7 (2/6)	
Good	40.4 (74/183)	42.4 (14/13)	37.5 (3/8)	38.5 (10/26)	
Fair	32.8 (60/183)	24.2 (8/13)	25.0 (2/8)	42.3 (11/26)	
Poor	8.7 (16/183)	18.2 (6/13)	12.5 (1/8)	11.5 (3/26)	
9 mo					0.0287
Excellent/very good	1.9 (3/186)	6.5 (2/13)	0 (0/8)	0 (0/3)	
Very good	15.3 (26/186)	18.1 (5/13)	33.3 (2/8)	8.7 (2/3)	
Good	42.5 (88/186)	48.4 (15/13)	33.3 (2/8)	21.7 (5/23)	
Fair	33.8 (64/186)	16.1 (5/13)	0 (0/8)	47.8 (11/23)	
Poor	5.6 (9/186)	12.9 (4/13)	33.3 (2/8)	21.7 (5/23)	
Body weight, mean (SD) (n/N), kg					
3 mo					
BL	101.4 (25.1) (242/244)	101.8 (26.7) (26/26)	112.2 (27.9) (5/5)	111.5 (34.8) (13/13)	0.4472
3 mo	101.3 (23.9) (215/244)	107.0 (24.9) (14/26)	109.7 (24.3) (3/5)	109.6 (37.1) (13/13)	0.4797
6 mo					
BL	100.5 (23.9) (253/253)	98.4 (22.6) (36/36)	118.2 (28.6) (8/8)	115.1 (36.3) (27/27)	0.0129
3 mo	100.1 (23.4) (214/253)	98.0 (21.4) (30/36)	118.4 (24.8) (8/8)	110.2 (32.1) (26/27)	0.0789
6 mo	100.0 (23.2) (140/253)	96.1 (19.9) (21/36)	115.6 (34.4) (8/8)	120.8 (34.8) (21/27)	0.0013
9 mo					
BL	100.8 (23.5) (167/166)	99.6 (24.4) (36/36)	101.5 (31.4) (6/6)	118.5 (36.3) (25/25)	0.0124
3 mo	100.4 (23.1) (144/166)	99.3 (23.9) (31/36)	101.6 (28.0) (6/6)	112.1 (32.6) (23/25)	0.2123
6 mo	100.3 (23.5) (120/166)	99.5 (23.9) (20/36)	100.3 (23.5) (6/6)	124.0 (30.9) (20/23)	0.0017
9 mo	100.4 (24.8) (120/166)	94.5 (13.9) (17/36)	117.5 (27.4) (15/23)	0.0669	
HbA1c, mean (SD) (n/N), %					
3 mo					
BL	8.15 (1.88) (242/244)	7.55 (1.88) (22/26)	7.96 (0.85) (5/5)	8.64 (1.78) (10/13)	0.5174
3 mo	7.93 (1.55) (215/244)	7.01 (1.40) (14/26)	7.30 (0.72) (4/5)	8.36 (1.55) (7/13)	0.2853
6 mo					
BL	8.03 (1.45) (253/253)	8.24 (1.50) (36/36)	8.34 (0.71) (7/7)	8.31 (1.47) (22/27)	0.8801
3 mo	7.87 (1.53) (214/253)	7.36 (1.38) (30/36)	7.02 (0.74) (6/6)	8.26 (1.39) (19/27)	0.1476
6 mo	7.76 (1.27) (140/253)	7.30 (1.38) (21/36)	6.55 (1.12) (4/4)	8.54 (1.34) (21/27)	0.0412
9 mo					
BL	7.95 (1.59) (167/166)	8.25 (1.77) (25/36)	7.72 (0.59) (6/6)	8.43 (1.88) (21/25)	0.5309
3 mo	7.91 (1.22) (144/166)	7.40 (1.22) (31/36)	7.40 (1.22) (6/6)	8.04 (1.52) (23/25)	0.0820
6 mo	7.77 (1.28) (120/166)	8.45 (1.04) (20/36)	7.00 (1.30) (6/6)	8.59 (1.29) (20/23)	0.0020
9 mo	8.07 (1.53) (120/166)	8.01 (1.44) (10/36)	7.00 (1.51) (4/4)	8.14 (1.49) (9/25)	0.6711

Between-group comparisons were made using one-way analysis of variance for continuous data and X2 test for categorical data.

Supported by: Sanofi US

1071

Overtreatment in elderly patients ≥ 75 years with type 2 diabetes and renal diseaseA. Penforis¹, J.-F. Blicke², B. Fiquet³, S. Dejager³;¹Jean Minjoz Hospital, Besançon, ²Strasbourg University Hospital,³Novartis Pharma SAS, Rueil Malmaison, France.

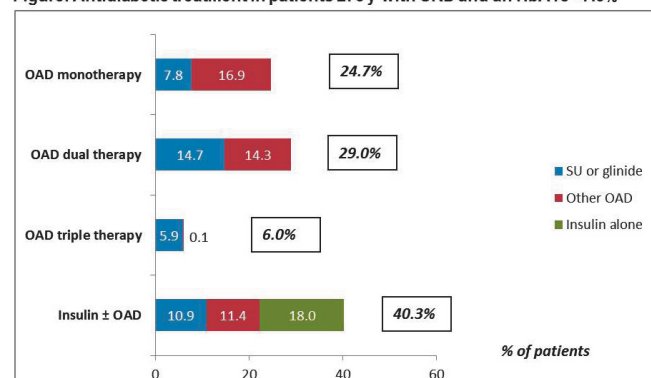
Background and aims: Few data exist regarding management of patients (pts) with type 2 diabetes mellitus (T2DM) over 75 years (y), who frequently have renal impairment (RI). This real-life study aimed to assess the treatment of T2DM in elderly patients with chronic kidney disease (CKD), a uniquely fragile population that poses specific challenges.

Materials and methods: Observational cross-sectional study: 3704 pts with T2DM were recruited by 968 physicians in France. Data from 980 diabetic pts ≥ 75 y with CKD are presented in this sub-analysis.

Results: Mean age was 81 y (range 75–101), 56% were male, with BMI 28.5 kg/m², while mean estimated glomerular filtration rate (eGFR) was 43 mL/min/1.73m². 20% of patients had severe RI (eGFR <30 mL/min/1.73m²; mean 22.5) and 71% had moderate RI (eGFR 30–60 mL/min/1.73m²; mean 43.7). 38% had seen a nephrologist. Mean HbA1c was 7.4% (60% had HbA1c $<7.5\%$ and 36% $<7\%$). They had long-standing T2DM (15.4 y), often complicated (macrovascular complications 49%, heart failure 20%, retinopathy 20%). Respectively, 96% and 78% had hypertension and dyslipidemia, almost all treated. They received a mean of 5.5 concomitant therapies for cardiovascular risk management. Antidiabetic therapy was oral-based for 51% of pts (40, 46 and 13% as single, dual or triple-drug therapy) and insulin-based for 49% (combined with ≥ 1 oral antidiabetic [OAD] in 59%). Treatment included metformin (47%), SU (26%), glinide (19%), α -glucosidase inhibitor (6%), DPP-4 inhibitor (31%) or insulin (49%). The type of agents differed across renal function (RF) strata: there was less use of metformin (70, 49 and 29% of pts, respectively, in normal RF, moderate or severe RI), SU (28, 28 and 19%) and DPP-4 inhibitors (38, 33 and 20%) and more use of glinides (11, 19 and 22%) and insulin (37, 46 and 65%) with declining RF. Metformin daily dose remained high (mean 1.9 g/d; >2 g/d for 25%) across all degrees of RI. Metformin was stopped/reduced at the end of the visit in only 25% of the metformin-treated pts overall and 40% of those with severe RI. 60% (n=579) of these older pts with overt RI and multiple comorbidities (nearly half with macrovascular disease) had an HbA1c level $<7.5\%$ (mean 6.7%) while being still quite intensively treated (Figure). Only $\frac{1}{4}$ were receiving oral monotherapy and 69% were receiving insulin secretagogues and/or insulin.

Conclusion: In clinical practice, most elderly T2DM pts with CKD appeared to be over-treated, putting them at high risk for severe hypoglycaemia. RI was insufficiently taken into account when adjusting anti-diabetics; at odds with current guidelines, 30% and 19% of elderly pts with severe RI were receiving metformin and SU, respectively.

Figure: Antidiabetic treatment in patients ≥ 75 y with CKD and an HbA1c $<7.5\%$



CKD, chronic kidney disease; OAD, oral antidiabetic drug; SU, sulphonylurea; y, years

Supported by: Novartis

1072

Patient-reported outcomes and costs associated with insulin and non-insulin therapies for type 2 diabetes in five European countriesS. Nuhoho¹, J. Vietri², G. Isherwood³, M. Worbes-Cerezo⁴;¹Janssen-Cilag A/S, Birkerød, Denmark, ²Kantar Health, Milan, Italy,³Kantar Health, Epsom, ⁴Janssen Cilag Limited, High Wycombe, UK.

Background and aims: The progressive nature of type 2 diabetes (T2D) often requires escalation of therapy for glycemic control and to protect against complications, but the consequences of different management strategies on patient well-being are not well documented. This study was conducted to describe patient outcomes by medication regimen.

Materials and methods: Data came from the 2013 5EU National Health and Wellness Survey, representative of adults in France, Germany, Italy, Spain, and UK in terms of age and gender. Respondents were categorized into four groups by use of antihyperglycemic medications: 1 non-insulin medication, 2 non-insulin medications, ≥ 3 non-insulin medications, and therapy including insulin. Outcome measures included the SF-36v2, Work Productivity & Activity Impairment questionnaire, and 6-month self-reported healthcare use. Indirect costs were estimated using work impairment and wage statistics, and direct medical costs estimated using reported medical visits and unit costs. Groups were compared using ANOVA with pairwise t-tests and chi-square test for continuous and categorical variables, respectively.

Results: Of 2,894 respondents included in the final sample, 68% were male and 32% were employed. Respondents using insulin had the lowest average scores for mental health component summary (44.9) by >2 points, physical component summary (40.9) by 3.8 points, and SF-6D health utility (0.63) by 0.05 points. Twice as many respondents using insulin (24%) reported ≥ 2 hypoglycemic events requiring assistance per year as non-insulin users (5.6–11.9%). The average rating of activity impairment was 1.3 times as high in insulin users (46.5%) as in non-insulin users (32.0–34.2%), with similar increases in ratings of work impairment among the employed subsample using insulin (32.4%) vs other employed respondents (21.3–24.9%). Healthcare use was most frequent in respondents using insulin, with a mean 11 total provider visits vs 8 during the same period for other groups. Emergency visits (mean 0.4) and hospitalizations (mean 0.3) were also most frequent among insulin users. Estimated annual indirect costs were higher in the insulin group than in non-insulin mono- and dual-therapy patients, while estimated direct costs were higher among insulin users than any other group (Table). All presented differences $p < 0.05$.

Conclusion: T2D patients using insulin experience greater burden in health-related quality of life, impairment to work and activities, and use more healthcare than patients managed without insulin. Therapies that delay insulin initiation may potentially offer better quality of life and work productivity to patients and provide savings to the healthcare system, though confounds such as duration of diabetes may have affected results. Further research comparing insulin-based and non-insulin management strategies is required.

	Non-insulin therapies						Insulin therapies	
	Monotherapy		Dual therapy		Triple or greater therapy		Mean	SD
Annual costs	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Indirect								
Absenteeism	€ 1,533	€ 4,626	€ 1,539	€ 5,108	€ 2,008	€ 7,371	€ 2,107	€ 5,558
Presenteeism	€ 3,353*	€ 5,004	€ 3,110*	€ 4,757	€ 3,294	€ 4,742	€ 4,413	€ 5,843
Total indirect	€ 4,883*	€ 7,111	€ 4,650*	€ 7,305	€ 5,302	€ 8,667	€ 6,520	€ 8,706
Direct	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Physician visits	€ 588*	€ 721	€ 579*	€ 594	€ 586*	€ 634	€ 853	€ 1,010
Emergency room	€ 52*	€ 193	€ 36*	€ 151	€ 23*	€ 96	€ 94	€ 319
Hospitalization	€ 460*	€ 1,466	€ 326*	€ 1,314	€ 336*	€ 1,099	€ 929	€ 3,797
Total direct	€ 1,099*	€ 1,856	€ 941*	€ 1,713	€ 945*	€ 1,402	€ 1,876	€ 4,349

* $p < 0.05$ vs. insulin.

Supported by: Janssen Pharmaceutica NV

1073

Randomised trial of a type 2 diabetes community case worker intervention reduces hospitalisations and emergency visits over 3 years in a collaborative care modelR.O. Yeung¹, Y. Zhang¹, A.O. Luk¹, Y. Cheung², R. Ozaki¹, H. Chung¹, J.C.N. Chan¹, W. So¹;¹Medicine, Chinese University of Hong Kong,²Ma On Shan Family Medicine Clinic, Hong Kong.

Background and aims: Collaborative care between family medicine doctors and specialists with periodic review at a diabetes centre reduced major clinical outcomes and mortality compared to management by family doctors alone. Further, increased contact time with support staff who promoted self-management and reduced negative emotions has improved metabolic control and reduced acute health care utilization. We hypothesized that provision of a community care worker (CCW) in the setting of collaborative care further improves metabolic control and reduces acute health utilization in patients with type 2 diabetes.

Materials and methods: In a collaborative care model, patients with type 2 diabetes attending a community-based family medicine clinic underwent annual comprehensive assessment (CA) at a specialist diabetes centre for risk stratification with personalized report and decision support using the web-based Joint Asia Diabetes Evaluation portal and were randomized to either additional support by a trained CCW (JADE+CCW) or JADE alone (JADE). Guided by the personalized report, the CCW counselled patients after the medical consultation to reinforce treatment adherence, self-care, and provide emotional support. Using intention-to-treat analysis, primary outcome was change in glycemic control after 3 years. Secondary outcomes included cardiometabolic control (changes in blood pressure and LDL-C), as well as acute health care utilization as measured by number of hospitalizations, total length of stay (TLOS), and number of emergency visits over 3 years. Metabolic control was measured at CAs. Hong Kong's public health care system services the majority of the population, so utilization measures were retrieved from the Health Authority database that captured all visits to public medical institutions within 3 years of enrolment. Negative binomial regression was used to ascertain the incident rate ratio (IRR) of hospitalization and emergency visits between groups, as well as rate ratio (RR) of TLOS.

Results: Of 661 patients recruited, 332 were randomized to JADE and 329 to JADE+CCW. At entry, 46% were male, with mean±SD age of 60.3±10.9 years, diabetes duration of 6.8±6.0 years, and HbA1c of 6.8±1.1%. The median number of CAs was 4 in both groups over 3 years, and those in the intervention group saw the CCW 12.2±4.3 times. After 3 years, HbA1c deteriorated in both groups (JADE 0.44% (95%CI 0.32–0.55%) and 0.48% (95%CI 0.37–0.70) in JADE+CCW (P=0.635 between groups)), whereas LDL-C improved in both groups. Systolic blood pressure improved in JADE+CCW from baseline (133.1 to 129.5mmHg, P<0.001) but did not in JADE (131.8 to 131.4mmHg P=0.406). In JADE, 44% had ≥ 1 hospitalization with a mean TLOS of 7.1±23.1 days and 50% had ≥ 1 emergency visit. The respective figures in JADE+CCW were 35%, 3.3±11.2 days and 43%. After adjusting for age, sex, diabetes duration, and risk level, the IRR (95% CI) in JADE+CCW was 0.77 (0.61–0.97) for hospitalization, 0.68(0.55–0.84) for emergency visits, and TLOS was 0.45(0.37–0.54) times as long compared to JADE.

Conclusion: The use of a trained CCW within a collaborative care setting reduced acute care utilization in patients with type 2 diabetes. These findings highlight the importance of using knowledge transfer and team-based management to make quality diabetes care more accessible and sustainable.

Supported by: Asia Diabetes Foundation

PS 091 Tailored diabetes care

1074

Tailored support for type 2 diabetes patients with a first acute coronary event after discharge from hospital: results of a RCT in primary care

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Background and aims: Type 2 diabetes mellitus (T2DM) patients with a recent acute coronary event (ACE) might experience decreased quality of life and increased distress. The first period after discharge is a critical time. We aimed to evaluate the effectiveness of a tailored supportive intervention on diabetes related distress, psychological well-being and clinical variables in T2DM patients and a recent first ACE.

Materials and methods: In this randomised controlled trial, the intervention group received a tailored supportive intervention based on the results of focus groups with patients and their partners. A diabetes nurse visited the T2DM patients at home within three weeks after discharge from hospital after a first ACE, and two weeks and two months later. From a list of ten topics, three were chosen by the patient and discussed with the patient and his/her partner, based on a protocol and with the use of a patient's handbook with assignments and homework. The attention control group received a consultation by telephone. Outcome variables were measured after discharge and five months later, using validated questionnaires: diabetes-related distress (PAID) well-being (WHO5), health status (EQ VAS), anxiety and depression (HADS). HbA1c, blood pressure and lipids were retrieved from the general practitioners' medical records. Differences between groups in change over time were analysed according to the intention-to-treat principle, using ANCOVA. The Holm-Bonferroni correction is used to adjust for multiplicity.

Results: Of the 201 randomised patients, 81 patients from the intervention group (age 65.8±9.3 yrs, 76.5% male) and 80 from the control group (age 65.6±9.4 yrs, 75% male) completed both baseline and follow-up questionnaires. All patients experienced low distress after discharge from hospital (8.7±11.8), which did not increase during the five months after discharge. Low levels of anxiety (4.1±3.8) and depression (3.7±3.5) were experienced at baseline and did not increase after five months. Baseline well-being (Intervention group: 60.6±27.4; control group: 58.2±25.5) and health status (Intervention group: 71.2±13.4 and control group: 68.8±16.2) were less favourable in both groups. A significant intervention effect was found for health status (effect size=0.35; p=0.007) and a trend was found for well-being (effect size=0.22; p=0.061). This corresponds to a small to moderate statistical and clinical difference in health status and in well-being between the two intervention and control group. From the patients in the intervention group (n=48) who received cardiac rehabilitation as well, 62% rated the intervention as having added value. Especially the individualised and timely character of the intervention was appraised.

Conclusion: This tailored intervention significantly improved the health status of T2DM patients shortly after discharge from hospital because of their first ACE. It did not influence the fortunately low levels of diabetes related distress, anxiety or depression in this group of patients.

Clinical Trial Registration Number: NCT01801631

Dutch Diabetes Research Foundation (no: 2009.70.)

1075

Improvements of diabetic control in a diabetes day clinic compared to the classic out-patient approach

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Background and aims: Diabetic day clinics allow patient - focused, structured and extensive education, improving knowledge about the nature of diabetes, its complications, and therapy options. In a small group environment patients are able to ask questions, share their problems and hear other people's experiences. More frequent and precise pharmacological interventions are an additional benefit of diabetic day clinics. With acquisition of knowledge and skills necessary to control diabetes, a positive attitude of patients towards their illness develops, allowing better diabetes control on the long-term. The aim of this research is to show the superiority of a combined struc-

tured approach in diabetes day clinics in the treatment of adult patients with diabetes in comparison to periodical visits in out-patient clinics.

Materials and methods: A total of 280 adult diabetic patients participated in this study, 140 patients were treated in the diabetes day clinic, and 140 controls were treated as out-patients. There was no significant difference between groups considering age, sex, diabetes type, and diabetes duration at study start. The study group underwent structured theoretical and practical education about diabetic nutrition, weight control, physical activity, acute and chronic diabetic complications and pharmacological management. They received psychological assistance, and training in specific skills in the diabetes day clinic. The control group received standard care in the out-patient clinic. Routine biochemistry relevant to glycemic control (e.g. fasting and postprandial glycemia, HbA1c, lipidogram) was determined, and - if necessary - therapy was adjusted in all patients. BMI and routine biochemistry were repeated after 3 months. Statistical analysis was performed by using Student's t-test for numerical data, and chi-square test for qualitative data.

Results: In the study group, both fasting (10.68 ± 3.89 vs. 8.34 ± 2.49 mmol/L, $p < 0.0001$) and postprandial blood glucose (12.79 ± 4.97 vs. 10.70 ± 4.43 mmol/L, $p < 0.0001$) were significantly improved during treatment in the diabetes day clinic, consecutively there was a statistical significant reduction in HbA1c at the first control visit performed after three months (9.08 ± 2.33 vs. 7.2 ± 1.51 , $p < 0.0001$), body mass index (30.33 ± 5.80 vs. 30.11 ± 5.33 kg/m², $p = 0.0029$), and improved HDL cholesterol (1.21 ± 0.36 vs. 1.33 ± 0.36 mmol/L, $p = 0.0003$). In the control group, there was no statistical significant difference in controlled parameters noticed on control visits. Additionally, patients in the study group had significant lower values of fasting (8.34 ± 2.49 vs. 8.92 ± 3.03 mmol/L, $p = 0.03$), and a significant more profound drop in HbA1c levels (-1.84 ± 0.15 vs. $+0.09 \pm 0.25$, $p < 0.0001$) in comparison to controls after three months of follow up.

Conclusion: Diabetic day clinic provide a superior way to intervene in glyemic control, offering better chances for life style changes (reflected in a significant weight loss) and a better glycemic control.

1076

Relation between perception of professional care provision and self-care activities in people with diabetes: Japanese DAWN2 subsample analysis

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Background and aims: It has not been studied whether the perception of people with diabetes (PWD) that they received care by a healthcare professional was associated with adherent self-care activities in PWD. The aim of this analysis was to investigate the association between the perception of PWD that they received diabetes care by a healthcare team and the diabetes self-care activities practiced by PWD and glycemic control using Japanese sub-sample data from the second Diabetes Attitudes, Wishes and Needs (DAWN2) study.

Materials and methods: The DAWN2 study was a multi-national questionnaire-based online survey of perceptions about healthcare provision for benchmarking and sharing of clinical practices to improve diabetes care in 17 countries. In Japan, a total of 508 PWD participated in the survey. PWD were asked whether they experienced healthcare provision (measuring HbA1c, blood pressure, lipid profile, etc.) during the past 12 months. Diabetes self-care activities were assessed using the Summary of Diabetes Self-Care Activities (SDSCA)-6 scores, representing the mean number of days that PWD performed specific self-care activities in the past 7 days. Statistical comparison was performed using the Mann-Whitney U test.

Results: The mean age of PWD was 54 years, and 57.9% were male. The mean duration of diabetes was 12.4 years and the mean HbA1c was 6.8%. With regard to healthcare provision, 91.3% of PWD recognized that they were provided with "measuring HbA1c level," and 71.9% of PWD recognized that they were provided with "measuring weight." In contrast, "examining feet" (15.7%) and "asking if having been anxious or depressed" were not frequently recognized (12.2%). PWD were more likely to practice "taking all diabetes medications exactly as previously agreed with a healthcare professional" (mean: 6.1 days) and "eating healthily" (4.1 days), and were less likely to practice "testing blood sugar" (2.6 days) and "checking feet" (1.4 days). PWD who recognized that they were provided healthcare provision, such as "examining feet" and "asking about the amount of physical activity," showed a significant difference in the days of practicing the related self-care activity compared to PWD who didn't experience the healthcare provision. ("examining feet": 2.0 days vs. 1.3 days, "asking about the amount of physical activity": 4.2 days vs. 2.5 days.) PWD who experienced "examining feet" also showed a significant difference

in the days of practicing not only "checking feet" but also "eating healthily" and "testing blood sugar the number of times recommended by a health care provider" compared to those who didn't. HbA1c was significantly lower in the group who practiced 5 days or more of the following self-care activities: "eating healthily" and "being physically active" compared with the group who practiced less than 5 days of those self-care activities.

Conclusion: The findings of this analysis suggest that PWD who reported to have received sufficient healthcare provision practiced certain self-care activities more often, and that certain diabetes care provisions may have a favorable effect on all-around self-care activities. PWD who practice self-care activities more often may achieve better HbA1c as a treatment outcome.

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Shared decision making in type 2 diabetes using the Diabetes Medication choice Decision Aid: preliminary results from a cluster-randomised trial

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Background and aims: Patient involvement in healthcare decisions is advocated in existing type 2 diabetes guidelines and can improve the quality of care. Decision aids (DAs) are tools that facilitate the shared decision making (SDM) process. In a cluster-randomised clinical trial we compared the efficacy of the Diabetes Medication Choice DA with usual care in patients with type 2 diabetes in Greece.

Materials and methods: Practices, matched by setting and level of care were randomly allocated to use of the DA or usual care to support decision making for choice of antidiabetic medications. Eligible patients required treatment intensification (HbA_{1c} 7.5-10%) and had more than one available treatment options. The trial comprised an initial clinical encounter and two follow-up visits. Immediately after the initial encounter, we assessed the quality of the decision making process by a modified 13-item Decisional Conflict Scale, patient's knowledge about antidiabetic medications, and patient's and clinician's satisfaction.

Results: We enrolled five practices (N=101) to the DA arm and four practices (N=103) to the usual care arm, between May 2013 and February 2014. Patients' baseline characteristics were equally balanced between the two arms. Patients in the decision aid arm had better (lower), albeit not significantly, scores in overall decisional conflict (mean difference 7.0; 95% CI -8.1 to 22.2), and its subscales (Table). Knowledge transfer was high in both groups (mean difference 2.4%; 95% CI -16.0 to 20.7). Patients allocated to the DA and standard practice were equally satisfied. In most cases, physicians found the DA useful and reported that its use and integration in their clinical setting was easy. They also appeared willing to use a similar DA for patients with other chronic conditions.

Conclusion: Our results are similar to findings from trials that assessed the Diabetes Medication choice DA in US. Promoting patient-centered care through a DA for type 2 diabetes in Greece was positively accepted by clinicians and patients. Further research is needed to determine the DA's impact

on care experience and patient-oriented outcomes on populations with different background sets of values and preferences.

Outcomes	Decision Aid (N=101), Mean [95 % CI]	Usual care (N=103), Mean [95 % CI]	MD* [95% CI]	p-value	ICC
DCS Overall†	17.9 [5.5, 30.3]	25.0 [10.4, 39.5]	7.0 [-8.1, 22.2]	0.31	0.32
DCS Informed subscale†	21.0 [2.7, 39.4]	34.6 [13.0, 56.2]	13.6 [-8.8, 36.0]	0.19	0.28
DCS Support subscale†	19.2 [5.0, 33.4]	22.4 [5.7, 39.1]	3.2 [-14.2, 20.5]	0.68	0.31
DCS Effective subscale†	14.7 [5.4, 24.0]	19.5 [8.6, 30.4]	4.8 [-6.6, 16.1]	0.35	0.21
Patient Knowledge Transfer	68.4% [53.3, 83.4]	70.7% [53.0, 88.4]	2.4% [-16.0, 20.7]	0.77	0.24

MD: mean difference; ICC: intra-cluster correlation coefficient; DCS: Decisional Conflict Scale

*Usual care versus Decision Aid; positive values for DCS outcomes favour the Decision Aid

†Lower DCS scores indicate better outcome

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Group-based self-management support leads to more adequate exercise behaviour in recently diagnosed type 2 diabetes patients

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Background and aims: Adequate self-care behaviours and lifestyle changes are considered essential, though challenging elements in the management of type 2 diabetes mellitus (T2DM). Previous research indicated that illness perceptions of patients and partners are important precursors for health behaviours. In addition, interventions based on the Common-Sense Model of Self-Regulation (CSM) seem effective in enhancing health outcomes in various (chronic) conditions. We therefore developed a group-based self-management support programme for recently diagnosed T2DM patients (1-3 years post-diagnosis) and their partners, based on the CSM. In this study, we want to investigate the short and long-term effectiveness of this new course on self-care behaviours in T2DM.

Materials and methods: Randomised controlled trial with a pre-test (T0), post-test (T1) design and follow-up after six months (T2). T2DM patients were selected on the basis of medical records of participating general practices in different regions in the Netherlands and, after informed consent, randomly allocated to the intervention (four course sessions) or attention control (single information meeting) condition. Self-care was assessed with three items of the revised Summary of Diabetes Self-Care Activities (SDSCA) measure, assessing exercise, diet (both 0 - 7 days) and smoking (yes/no) during the past week. Exercise and diet were dichotomised into adequate (≥ 5 days) and non-adequate behaviours. Effectiveness on self-care was established by logistic regression analyses adjusting for demographic characteristics (age, gender and educational level) and pre-test scores on self-care.

Results: A total of 167 patients participated in the study: 81 in the intervention and 86 in the control condition. 146 patients returned the T1 questionnaire (drop-out rate 13%). Participating patients had an average age of 64 years (SD = 10.10, 27 - 83 years) and 56% were male. At baseline, 43% of the patients reported adequate exercise, 77% adequate dietary and 82% non-smoking behaviours. Preliminary results showed a significantly higher proportion of self-reported exercise behaviours in the intervention group (47%) at T1 (OR = 2.42, CI = 1.07 - 5.50), compared to the control group (32%). No significant differences between the intervention and control group were found on (changes in) reported dietary (76% vs 72%) and non-smoking behaviours (87% vs 74%) at T1.

Conclusion: In the short term, a self-management support programme based on the CSM appears to be effective in enhancing adequate (self-reported) exercise behaviours in recently diagnosed T2DM patients. Results on long-term effectiveness of the course are currently investigated and will be presented during the conference.

Clinical Trial Registration Number: NTR3302

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Diabetes care process performance using the alphabet strategy compared to the national audit data: practice of evidence based medicine (POEM) 2013

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Background and aims: The Alphabet Strategy is a diabetes care checklist based on a mnemonic: A for advice with regard smoking cessation, ideal weight attainment, regular exercise; B for blood pressure; C for cholesterol profile and creatinine care; D for diabetes glycaemic control; E for yearly eye exam; F for regular year foot exam; and G for guardian drugs with regard to aspirin, ACE inhibitors, and statins. Such a management strategy has been demonstrated to be effective in ensuring performance of care processes and attainment of diabetes target parameters. We performed an audit of the notes of all patients with Type 2 diabetes attending the diabetes outpatient department using the Alphabet Strategy as an audit template to determine adherence to the checklist. We compared these results to the UK's recently published National Diabetes Audit 2011/12.

Materials and methods: The Diabetes Outpatient Clinic register was consulted for the names of patients currently attending the department. Relevant demographic and diabetes data were extracted from information attained at most recent clinic visit. Case notes, electronic letters and the computerised pathology reporting system were reviewed for outstanding care processes. Care performance within 15 months of the most recent clinic visit was registered as positive documentation. Data collection was performed over three months between October to end of December 2013. Statistical analysis was performed using chi-squared test.

Results: Data was available from 551 patients. Mean age of the whole cohort was 63 years. 18.7% of people were more than 74 years of age. Men comprised 59.2% of the study group, and as a group were of similar average age to females at 63 years. Compared to the NDA, POEM achieved better performance of care processes except for annual foot exam. For target process achievement, POEM achieved better control in total cholesterol and target blood pressure, but fared worse in glycaemic control.

Conclusion: The use of the Alphabet Strategy continues to help achieve performance of essential diabetes care processes.

Care process recorded	Care process performed % of cohort		p value
	POEM 2013	NDA 2011/12	
Smoking	96.9	85.9	0.002
BMI	94.1	91.3	0.320
Blood pressure	100	95.8	0.036
Total cholesterol	100	92.4	0.004
Creatinine	99.6	93.8	0.016
Urine albumin creatinine ratio	80.9	77.9	0.470
HbA1c	100	91.3	0.002
Eye screening	97.3	-	
Foot exam	84.0	87.0	0.372
Eight care processes (minus eye exam)	65.0	62.6	0.620

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Cost-effectiveness of centralised and partly centralised care compared to usual care for patients with type 2 diabetes

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Background and aims: Due to an ever increasing number of type 2 diabetes patients, innovations to control the increasing health care use and costs are needed. Results of diabetes care programs on the costs or (cost-) effectiveness are heterogeneous. The aim of this study is to compare the cost-effectiveness of two diabetes care models with usual care for type 2 diabetes patients from the societal perspective.

Materials and methods: An economic evaluation was performed alongside a clinical trial. In two distinct regions of the Netherlands, two diabetes care models were implemented with different levels of centralized organizational structures. One of them was centralized care (CC) with a central organization and coordination of the care between all care providers and the use of a central database. Patients receive an annual extended diabetes assessment at the Diabetes Care Centre, in addition to the care by patients' general practitioner (GP). GPs receive feedback about their performance. Partly centralized care (PC) focuses on adherence to type 2 diabetes guidelines. An online clinical database is used to monitor mean values of risk factors. All assessments were performed in patient's GP practice. Usual care (UC) has a decentralized organisation structure and patients' GP is responsible for the diabetes care. Clinical outcome measure was risk of a coronary heart disease (CHD) calculated with the UKDPS risk engine. Cost-effectiveness analysis was performed from the societal perspective comparing patients receiving CC (n=313), PC (n=293) and UC (n=485) during one year of follow-up. Missing costs and effects data were imputed using multiple imputation. Differences in costs, effects and cost-effectiveness between the diabetes care groups were analysed using bootstrapping techniques.

Results: Differences in changes in CHD risk over 12 months of follow-up between the three groups were statistically insignificant and clinically irrelevant. Compared to UC, health care costs during the follow-up period were lower in CC (-1300 (95% CI: -2300 to -570)) and PC (-960 (95% CI: -1890 to -100)). Costs from the societal perspective showed the same trend, although not statistically significant.

Conclusion: Clinical outcomes did not differ between the different care models. Lower health care costs were observed in (partly) centralized care compared to usual care, mainly due to substitution of secondary health care use by primary health care use. This suggests that centralizing the diabetes care results in equal outcomes at lower health care costs.

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The effectiveness of the Diabetes Shared Care Program for diabetes-related avoidable hospitalisations in Taiwan: a nationwide population-based study

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Background and aims: The Diabetes Shared Care Program was implemented in 2001 by Taiwan's Ministry of Health and Welfare to improve the quality of diabetes care. The efficacy of this program is unknown and we aimed to evaluate whether Diabetes Shared Care Program participants had lower frequency of avoidable hospitalizations in Taiwan.

Materials and methods: We collected nationally representative data from Taiwan's National Health Insurance Research Database. The dataset comprised 120,000 patients who were newly diagnosed with type 2 diabetes in 1999. The analysis included the patients' follow-up data until December 31, 2011. We designed a case-control study consisting of patients with avoidable hospitalizations as cases and selected two age-, gender-, and avoidable hospitalization duration-matched controls by risk-set sampling. We further compared the hypoglycemia risk between Diabetes Shared Care Program participants and non-participants. We used the conditional logistic regression to estimate the crude and adjusted odds ratio (OR).

Results: A total of 2,377 avoidable hospitalizations cases and 4,754 matched-controls were identified. After adjusting for potential confounders in the stratified analyses, Diabetes Shared Care Program participants had a significantly lower frequency of all diabetes-related avoidable hospitalizations (OR, 0.15; 95% CI 0.13-0.17). A similar trend was found for short-term complications (OR 0.13; 95% CI 0.08-0.20), long-term complications (OR 0.13; 95% CI 0.11-0.16), uncontrolled diabetes (OR 0.21; 95% CI 0.15-0.30), and lower-extremity amputations (OR 0.06; 95% CI 0.03-0.13).

Conclusion: The Diabetes Shared Care Program decreased the frequency of all diabetes-related avoidable hospitalizations in Taiwan.

PS 092 Screening and risk factors for gestational diabetes mellitus

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Screening for gestational diabetes mellitus in resource constrained settings

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Background and aims: The International Association of Diabetes in Pregnancy Study Groups (IADPSG) criteria for screening for gestational diabetes mellitus (GDM) requires 3 venous samples drawn in the fasting state. This is difficult in resource-poor settings for two reasons: women find it difficult to come to the clinic in the fasting state and also obtaining three blood samples is difficult due to limited resources. We therefore assessed the usefulness of a non-fasting capillary blood glucose (CBG) as a preliminary screening test to reduce the number of women who would need to be referred for a definite diagnostic test done in the fasting state.

Materials and methods: Pregnant women (n=1031) attending antenatal clinics in Tamil Nadu in Southern India underwent a CBG test 2 hours after a 75 g glucose load administered irrespective of time of last meal (non-fasting). Participants were requested to come back within the next 2 to 3 days for a fasting OGTT, and both IADPSG and WHO (1999) criteria were used for diagnosis of GDM. The optimal sensitivity and specificity of the non-fasting 2 hour CBG value to identify GDM diagnosed by IADPSG and the WHO (1999) criteria were determined.

Results: A non-fasting 2 hour CBG value of 126 mg/dl (6.9 mmol/l) had sensitivity and specificity of 64.6% and 63% respectively to identify GDM diagnosed by IADPSG criteria, but 40% of all pregnant women would need to be referred for the fasting OGTT. For the WHO 1999 criteria, the CBG cutpoint of 144 mg% (7.9 mmol/l) had a sensitivity and specificity of 87.9% and 86.9% respectively and only 19.1% of the women would have to be referred for the fasting OGTT.

Conclusion: A 2 step screening procedure for GDM using a non-fasting 2 hour CBG as the initial screening test and use of WHO (1999) criteria would help to minimize the number of women who would need to be referred for a diagnostic OGTT in the fasting state and thus make screening for GDM feasible in resource constrained countries.

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Gestational diabetes: what is the impact of the adoption of the criteria of IADPSG?

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Background and aims: We have always performed a systematic screening for gestational diabetes (GDM). The recommendations made in 2010 by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) give the possibility for each country to choose whether the screening should be universal or selective on the basis of risk factors assessment (adjusted according to the prevalence of diabetes in the population). We have chosen to continue systematic screening. The aim of our study was to assess the level of risk in our screened population, and the maternal-fetal morbidity associated with GDM depending on the presence or absence of risk factors

Materials and methods: We retrospectively analyzed the data of all pregnant women admitted in day-hospital in the service of our hospital between June 1, 2010 and December 31, 2012 for screening for GDM. We conducted a systematic screening between 24 and 28 weeks of gestation by OGTT with 75 g glucose (IADPSG criteria). We evaluated the frequency of risk-factors: Age \geq 35 years, BMI \geq 25 kg/m², history of diabetes in first degree relatives, previous GDM or macrosomic child. We compared the incidence of maternal and

fetal complications between the group with risk-factors (RF +) and the group without risk-factors (RF -).

Results: Of 1680 women screened, 330 had a GDM, an estimated prevalence of 19.6%. Among them 52 (15.8%) showed no RF : average age 28.3 years, mean BMI 23.6 kg/m²; and 278 (84.2%) had at least one RF : 128 (46%) had age \geq 35 years, 208 (74%) BMI \geq 25 kg/m², 139 (50%) a history of GDM, 70 (25%) a history of macrosomia and 35 (12%), family history of diabetes. The maternal-fetal prognosis of the group without RF compared to the one with RF only differs in macrosomia: 7.3% vs 15.6% ($p < 0.05$), preeclampsia 0% vs 6.2% (NS), preterm (< 37 weeks) 16.6% vs 13.2% (NS), cesarean 32.2% vs 37.6% ($p = 0.44$), insulin 63% vs 49% ($p = 0.19$).

Conclusion: Our population has a high prevalence of GDM. It is diagnosed in a non-negligible population without RF. There is no significant difference in the maternal-fetal morbidity between the groups with and without RF, so it seems appropriate to maintain a systematic screening.

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National guidelines for gestational diabetes mellitus (GDM) screening in Italy: application and effectiveness

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Background and aims: Universal screening for GDM has been used in Italy for over 20 years; since 2012 the National Health Authority recommended GDM screening only for women with risk factors. According to these new Criteria, screening test (OGTT 75 g 2 hours) is recommended early in pregnancy (14–18 week) in women at high risk (HG: previous GDM; pre-pregnancy BMI ≥ 30 kg/m², glucose value at 1st visit between 100–125 mg/dL) and later in pregnancy (24–28 week) in women at medium risk (MR: pre-pregnancy BMI ≥ 25 and < 30 kg/m², age ≥ 35 years, previous macrosomia; positive family history of diabetes), while women at low risk (LR: no risk factors) are excluded from screening. The aim of the present study was to evaluate whether the new national guidelines (NGL), are correctly applied and the effectiveness of GDM diagnosis according to risk factor profile.

Materials and methods: We collected a cohort of 2552 Caucasian pregnant women (age 33 ± 5 years; family history of diabetes 18.2%; pre-pregnancy BMI 22.8 ± 4 kg/m²) consecutively screened for GDM according to NGL. For each category we recorded timing of screening test and prevalence of GDM. Finally, we compared GDM prevalence in this cohort with that one recorded in a historical cohort (years 2001–2003) of 3950 women with universal screening, this population being comparable with the most recent one (age 31 ± 5 yrs, family history of diabetes 18.1%; pre-pregnancy BMI 22.5 ± 3.7 kg/m²).

Results: Out of 98 LR women (3.8% of total), 2 (1.6%) underwent OGTT at early stage and the remaining 96 (98.4%) in the late period. MR women (n 2193) accounted for 86% and a late screening was performed in almost all cases (98.4%). The remaining 10.2% (n 261) included HR women who underwent early screening in only 7% of cases with 93% of them screened in the late period. GDM was diagnosed in 279 cases with a prevalence of 10.9%, i.e. 25.3% higher than previously reported in our population (8.7%; $P = 0.003$). Based on risk factory categories, GDM prevalence increased from 4.7% in LR, to 13.7% in MR women, and 29.8% in HR.

Conclusion: Our data show that the NGL recommendations are still poorly applied. Contrary to what recommended only a minority of HR women perform the screening test early in pregnancy, in spite of a 30% prevalence of GDM. Action has to be taken to improve application of new recommendations.

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Experiences of the providing for pregnancies complicated with IADPSG criteria

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Background and aims: Based on the results of the HAPO study IADPSG recommended in 2010 new criteria for diagnosing gestational diabetes (GDM). To our knowledge the new criteria might significantly increase prevalence of GDM. The aim of our study was to determine the result of the care of our IADPSG-GDM patients.

Materials and methods: Continuing the universal screening between 15th January 2009 and 15th March 2013 in the West Hungarian Region (Szekszárd district) a three-points 75 g OGTT (fasting, 60- and 120 min blood glucose values) were performed in 4658 pregnant women (age: 29.64 ± 5.55 yrs; mean \pm SD). GDM was diagnosed using new IADPSG criteria, too.

Results: 388 (WHO, 9.08 %) and 699 (IADPSG, 15.00%) resp., GDM cases were found. Women in both GDM groups - compared to healthy counterparts - were older (31.40 ± 5.19 vs. 29.51 ± 5.47 yrs [WHO]; 31.14 ± 5.13 vs. 29.35 ± 5.49 yrs [IADPSG]);, had significantly higher systolic blood pressure (121.38 ± 9.66 vs. 119.17 ± 9.33 mmHg; [WHO]; 121.62 ± 9.61 vs. 118.92 ± 9.26 mmHg [IADPSG]);. All-risk-scores of both GDM groups were significantly higher than that in the healthy women. According to both criteria 254 women had GDM. Using IADPSG criteria 104 WHO-GDM cases were lost, 435 new cases were found. There was no difference in the frequency of Caesarean section between healthy women and both GDM groups. Analysing data of 4900 newborn babies (71 twin pairs) no differences in the frequency of intrauterine death and congenital malformations between any groups could be proven. Babies born to IADPSG-GDM mothers had a bigger mean birth weight than newborn babies of healthy women (3413 ± 548 vs. 3327 ± 505 g) while no differences were found by babies of WHO-GDM mothers (3340 ± 537 vs. 3340 ± 511 g). The authors formed within IADPSG-GDM group treated /n:289/ (diet or diet and insulin) and untreated /n:409/ subgroup. The date in treated subgroup of IADPSG-GDM were: age: 32.02 ± 4.76 yrs, weight gain: 10.28 ± 5.54 kg birth weight: 3331 ± 531 . vs. the date in untreated subgroup of IADPSG-GDM were: age: 30.52 ± 5.30 yrs. weight gain: 13.18 ± 5.43 kg birth weight: 3470 ± 554 kg.

Conclusion: Analysing results of a universal screening using IADPSG criteria frequency of GDM probably doubles. In IADPSG-GDM the adverse pregnancy outcomes be prevented by complex pregnancy care.

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Age at menarche: a new risk factor for GDM?

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Background and aims: Earlier age at menarche has been associated with increased risk of type 2 diabetes (DM2) and obesity. As DM2 shares several common risk factors with Gestational Diabetes Mellitus (GDM), the aim of this study was to examine whether there is an association between age at menarche and GDM risk in a large cohort of pregnant women.

Materials and methods: Over a period of 10 years, at a tertiary hospital, 5390 pregnant women without known DM underwent a 100g OGTT in the third trimester of pregnancy. For GDM diagnosis the Carpenter and Coustan criteria were applied. Age, pre-pregnancy BMI, family history of DM2, parity, education status, smoking and age at menarche were recorded. For statistical analysis χ^2 , t-test were used; odds ratios (OR-95%CI) were calculated and stepwise logistic regression model was applied.

Results: GDM (n=2452) women presented with lower age at menarche compared to Normal (n=2938) pregnant women (12.9 ± 1.5 vs 13.1 ± 1.6 years, $p < 0.001$). Women with age at menarche equal to or more than 12 years were found to have a reduced risk of GDM (OR = 0.84 [95%CI = 0.76–0.94], compared to those with age at menarche less than 12 years. They also had lower BMI (25.1 ± 5.0 vs 26.4 ± 5.9 kg/m², $p < 0.001$). Further the prevalence of obesity (BMI > 30 kg/m²) was significantly lower in this group (14.1% vs 21.7%,

$p < 0.001$). The odds ratio for a woman with menarche age < 12 years to be obese was 1.7 [95%CI=1.5–1.9]. Additionally, the presence of a family history (FH) of DM2 was associated with earlier age at menarche (12.7 ± 1.5 vs 13.2 ± 1.6 years, $p < 0.001$). The OR for age at menarche < 12 years to have FH of DM2 was 1.4 [95%CI = 1.2–1.5]. Subsequently, a stepwise logistic regression model was applied where GDM was a dependent variable, and independent predictors were: age at menarche < 12 y, age > 25 y, positive FH of DM2 and BMI > 30 kg/m². The age at menarche remained an independent predictor of GDM when age and FH of DM2 were included in the model, but lost its significance when BMI was added in the model.

Conclusion: We showed that early age at menarche increases the risk of GDM, a finding which has not been reported. This association may be explained by the effect of obesity on both conditions, namely early menarche and GDM.

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Drinking alcohol before pregnancy induces the abnormal foetus development through oxidative stress-mediated metabolic disorders in maternal liver and pancreas

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Background and aims: Alcohol drinking during pregnancy poses serious health risks to the unborn child such as prematurity, low or high birthweight, fetal death and fetal alcohol syndrome. However, the effects of maternal ethanol consumption before pregnancy on the abnormal development of fetus and the association with the impaired glucose tolerance of mother are not fully understood.

Materials and methods: To this, the 6-week C57BL/6J female mice were fed with 5% ethanol-containing liquid diet for 2 weeks before pregnancy and examined the effects of ethanol pre-exposure on fetal development during a subsequent pregnancy.

Results: Here, we found that pregnancy or fertility rates were decreased in ethanol-fed mice, correlated with the delaying of eye formation and the formation of defective toe. Also, birth weight in postnatal 0 day (P0) of ethanol-fed mice was higher than those of pair-fed mice, but thereafter, in P14 and P21, growth retardation appeared in the child of ethanol-fed mice. The macrosomia phenomenon in ethanol-fed mice is strongly associated with the dysregulation of glucose metabolism and triglyceride accumulation in maternal liver during the pregnancy. Gut-derived serotonin (GDS) as well as hepatic inflammatory chemokines and cytokines are also markedly increased in ethanol-fed mice, subsequently followed by the alteration of glucose metabolism in the liver and pancreatic β -cells. Alcohol intake changed the expression of GDS-response receptors from Gs-GPCR to Gi or Gq-GPCR. The detrimental fetal development and impaired glucose metabolism by maternal pre-exposure to ethanol are strongly attenuated by the injection of 4-methyl-prazol, an inhibitor of CYP2E1.

Conclusion: Taken together, our results suggest that ethanol consumption before pregnancy is a major causing factor for the detrimental fetus development via maternal metabolic disorders, especially on the dysregulation of glucose or insulin metabolism in maternal liver and pancreatic β -cells.

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Adverse foetal outcomes in women at risk of gestational diabetes with normal OGTT: exploring the role of HbA_{1c} in risk prediction

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Background and aims: Screening for gestational diabetes (GDM) is currently based on performing oral glucose tolerance test (OGTT) in pregnant women with various risk factors such as high body mass index, ethnicity and previous macrosomia. Women who get diagnosed with GDM with a positive OGTT benefit from targeted antenatal care towards improving pregnancy outcomes. However pregnancy outcomes in those women at high risk for GDM but normal glucose tolerance at screening are not well documented. The aim of our study was to compare the fetal complication profile in women confirmed to have gestational diabetes (GDM) with women at risk of GDM

but normal OGTT and explore the potential utility of HbA_{1c} in predicting such fetal outcomes.

Materials and methods: The records of 40,943 deliveries performed at the regional maternity hospital over a 7 year period (2006 - 2013) were identified and reviewed. Women with pre-existing diabetes (type 1 or type 2) were excluded ($n=203$, 0.5%). Based on active identification of risk factors for GDM, OGTT was performed at around 28 week gestation ($n=8,542$). HbA_{1c} was performed simultaneously with the OGTT in line with the hospital policy. Women were categorized into (1) Low risk (i.e. those not screened for GDM with OGTT/HbA_{1c}) ($n=32,198$), (2) High risk with negative OGTT and HbA_{1c} < 42 mmol/mol ($n=7,420$), (3) High risk with negative OGTT and HbA_{1c} ≥ 42 mmol/mol ($n=157$) and (4) confirmed GDM ($n=965$).

Results: Women in high risk category with normal OGTT, especially those with HbA_{1c} ≥ 42 , had statistically significant ($P < 0.001$) and worst fetal outcomes (table 1). On logistic regression, the risk of fetal macrosomia was significantly higher in category 2 [OR 1.95 (1.57–2.42), $P < 0.001$] and category 3 [OR 3.7 (1.53–9.35), $P=0.004$], whilst for women with GDM it was a similar risk to that for women in low risk category. The risk of preterm delivery and still birth was also significantly higher in category 3 [OR 1.63 (1.02–2.61), $P=0.041$ and OR 4.87 (1.78–13.29), $P=0.002$ respectively] compared to the low risk group. The GDM group interestingly fared very similar to the low risk group, which could be largely attributed to the active intervention and follow up provided once diagnoses is established.

Conclusion: Women with risk factors for GDM but a negative OGTT, appear to be a high risk cohort for adverse fetal outcomes especially fetal macrosomia. A small but significant sub-cohort of these high risk women (1.8%) with elevated HbA_{1c} could be at a further risk of pre-term delivery and still birth. HbA_{1c} could potentially help in risk stratification of high risk women with negative OGTT. Such women may need risk reduction intervention including diet and life style measures in line with what is already offered for GDM.

Table 1: Fetal macrosomia (Birth weight > 4500 g), Pre-term delivery (Before 37 week gestation) and Still birth in women categorised into low risk (category 1), high risk (categories 2 and 3) and with GDM (category 4)

	Fetal macrosomia	Pre-term delivery	Still birth
Low risk (category 1)	1.3%	8.2%	0.5%
High risk with negative OGTT and HbA _{1c} < 42 mmol/mol (category 2)	2.6%	6.6%	0.3%
High risk with negative OGTT and HbA _{1c} ≥ 42 mmol/mol (category 3)	4.9%	12.7%	2.5%
GDM (category 4)	1.2%	9.4%	0.3%

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Beta cell death is not increased in pregnant women with gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) affects approximately 7% of all pregnancies in the United States. Although it is believed that insulin resistance and reduced β -cell function are the underlying causes of GDM development, changes in β -cell mass during GDM remain largely unknown. We have previously developed a novel biomarker assay for the detection of β -cell death in type 1 diabetes by measuring the levels of β -cell derived demethylated insulin DNA in the blood. This study is designed to evaluate the levels of β -cell death in normal subjects and in patients with gestational diabetes mellitus.

Materials and methods: Serum samples from women with gestational diabetes mellitus (GDM) were compared with samples from women with normal pregnancy (PRG), women at postpartum (PP), and non-pregnant (NP) women. Samples were analyzed for β -cell derived DNA as a biomarker of β -cell death. Postprandial glucose, insulin and c-peptide levels were also measured.

Results: Women with GDM showed higher glucose levels when compared with PRG (PRG=105.9 \pm 7.24 vs. GDM=201.7 \pm 6.96 mg/dL, $p < 0.0001$). Surprisingly, β -cell derived insulin DNA levels were lower in GDM when compared with PRG and NP groups (Demethylation index: PRG=0.19 \pm 0.09, GDM=0.009 \pm 0.0009, PP=1.06 \pm 0.78, and NP=0.05 \pm 0.047; GDM vs. PRG $p=0.0175$ and GDM vs. NP $p=0.0132$). This decrease in β -cell death was

associated with reduced insulin (PRG=18.69±2.22, GDM=4.05±2.34, PP=8.11±3.56, and NP=3.78±2.04 μ U/mL; ANOVA $p=0.0005$, PRG vs. GDM $p=0.01$, PRG vs. PP $p=0.05$, PRG vs. NP $p=0.01$) and c-peptide (PRG=3.36±0.64, GDM=0.24±0.10, PP=2.01±0.74, and NP=1.66±0.44 ng/mL; ANOVA $p=0.0042$, PRG vs. GDM $p=0.01$) levels.

Conclusion: This study shows, for the first time, lower β -cell death in women with GDM, suggesting that β -cell death is not a main contributor to the development of GDM.

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Genetic variants associated with type 2 diabetes and obesity better predict gestational diabetes than traditional risk factors

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Background and aims: There is increasing evidence that gestational diabetes (GDM) shares genetic risk with type 2 diabetes (T2DM) and that 50 % of women with prior GDM develop T2DM in the next few years. Recent meta-analyses have shown that T2DM risk variants may be associated with GDM through different mechanisms, including impaired β -cell function (CDKAL1, IGF2BP2, KCNQ1, KCNJ11, MTNR1B), insulin resistance (TCF7L2), and abnormal glucose utilization (GCK). So, in the present study we evaluated the risk of GDM development associated with genetic variants increasing the susceptibility for obesity and T2DM.

Materials and methods: Four hundred and seventy four pregnant women, including 103 diagnosed as having GDM according to the IADPSG criteria and 371 controls with normal glucose tolerance, were genotyped for 65 SNPs, identified in genome wide-associated studies as risk variants for T2DM and obesity.

Results: On the basis of genotype case-control differences we found an association between GDM and four obesity gene variants (BAT2 [rs2260000] - OR=2.07 [1.05-4.1], $p=0.03$; MTCH2 [rs10838738] - OR=1.97 [1.002-3.88], $p=0.045$; FAIM2 [rs7138803] - OR=1.95 [1.15-3.34], $p=0.012$; CRY2 [rs11605924] - OR=0.56 [0.34-0.92], $p=0.021$), and three T2DM risk variants (TCF7L2 [rs7901695] - OR=1.67 [1.16-2.4], $p=0.03$; SLC30A8 [rs11558471] - OR=1.67 [1.16-2.4], $p=0.007$; CDKAL1 [rs10946398] - OR=1.62 [1.02-2.56], $p=0.036$). In logistic regression model comprising all studied SNPs, adjusted for relevant clinical data, GDM was significantly predicted by BMI before index pregnancy (OR=1.15 [1.07-1.23], $p=5.2 \times 10^{-5}$) and 4 studied SNPs: CDKAL1 (rs10946398) - OR=1.67 (1.02-2.1), $p=0.04$; GCK (rs4607517) - OR=2.64 (1.31-5.3), $p=0.007$; ADRA2A (rs10885122) - OR=2.88 (1.36-6.1), $p=0.003$ and KCTD15 (rs29941) - OR=2.17 (1.2-3.9), $p=0.007$.

Conclusion: Our results suggest that genetic variants associated with T2DM and obesity better predict GDM than the patient's age, the history of previous births and BMI before pregnancy. The mechanisms related to GDM risk seem complex and comprise the effect of numerous genes involved in insulin secretion/function and glucose homeostasis.

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MTNR1B genetic variability is associated with gestational diabetes in Czech women

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Background and aims: The gene *MTNR1B* encodes a receptor for melatonin, the main regulator of sleep cycle and circadian rhythm. Melatonin receptors are expressed in the brain and also in human pancreatic β -cells. This finding implies that genetic variants in the gene might affect glucose tolerance. Meta-analysis confirmed that rs10830963 shows the most robust association. The aim of our study was to assess and compare the SNP rs10830963 in the Czech GDM patients and in control normoglycemic women without history

of GDM. We also aimed to study relations between the risk conferring allele G of the SNP and biochemical as well as anthropometric characteristics in both groups.

Materials and methods: Our cohort of subjects consisted of 880 women, 458 of them were diagnosed with GDM (age 34,0±6,12 years; BMI 24,3±4,93 kg/m²) and they met the 0.5-1.5 year interval after the childbirth. 422 were normoglycemic control women without history of GDM (age 34,8±15,09 years; BMI 23,7±4,18 kg/m²). We evaluated broad biochemical (3-hour oGTT, lipid profile, thyroid and steroid hormones, liver enzymes) and anthropometric (BMI, WHR, waist circumference, fat distribution, total body fat content) parameters in all participants. We used the ABI TaqMan SNP Genotyping Assays to genotype for rs10830963. Statistical analysis was conducted using the NCSS 2004 software.

Results: In accordance with available literature data, the risk allele G was in our study significantly more frequent in the GDM group (38.3 % vs 29.4 % in controls, test power 0.96; OR 1.49 CI 95% [1.22; 1.82] $pOR=0.0001$). Despite of higher frequency, the G allele in the GDM group was not associated with any markers of glucose metabolism nor with anthropometric data. In contrast, controls showed significant association of the allele G with fasting plasma glucose (median 4.95 mmol/l in GG genotype vs. 4.60 mmol/l in CC as well as in CC genotypes; $p=0.002$ and $p<0.001$, resp.) and also with postchallenge levels of glycemia during the 30., 60., and 90. min. of the oGTT. Fasting insulinemia was in controls similar across the genotypes, nevertheless, postchallenge insulin levels were higher in GG homozygotes compared with CC genotype in 90. and 180. min. of oGTT, the same results were observed in C-peptide concentrations. Consequently, Cederholm index of insulin sensitivity and HOMA β index were higher in CC homozygotes in comparison with risk GG genotype ($p=0.02$ and $p=0.05$, resp.).

Conclusion: Significantly higher frequency of the risk conferring allele G in GDM group indicates that *MTNR1B* SNP rs10830963 is involved in gestational diabetes mellitus in Czech women. However, the association of the SNP with glucose metabolism, which is obvious in healthy control group of women, disappears completely in women of similar age and BMI who have experienced GDM.

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PS 093 Gestational diabetes mellitus management

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Gestational diabetes mellitus and health care cost: short- and long-term association

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Background and aims: There is an extensive body of literature showing that gestational diabetes mellitus (GDM) diagnosed on WHO criteria is associated with maternal, ante- and neonatal complications. Yet, little is known about the health care cost associated with GDM beyond the period immediately after the delivery and maternity care cost associated with GDM on the International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria. In this paper, we assess the association of the GDM diagnosis on the IADPSG criteria with the health resource utilization during maternity care and in longer term, two to five years after the index pregnancy.

Materials and methods: Maternity care utilization was measured for a sample of 658 women drawn from the Atlantic Diabetes in Pregnancy (ATLANTIC DIP) collaborative network who have had pregnancy two to five years previously. Annual health care utilization two to five years after the index pregnancy was assessed for a sub-sample of 348 women who returned a follow-up questionnaire. Irish cost weights were applied to the health care resource utilization to assess the cost of care. The difference in resource utilization and cost between women with GDM and normal glucose tolerance in the index pregnancy was assessed in a series of uni-variate and multivariate analyses.

Results: The independent effect of GDM is approximately additional € 817.6 to the maternity cost and additional € 680.5 to the annual health care cost two to five years after the delivery. However, at the group/population level the effect might be higher due to inherent background differences of the women with GDM and NGT, especially worse body mass indicators. The excess in the maternity care cost is caused by higher likelihood of caesarean section and neonatal unit admissions in women with GDM. For the follow-up health care cost, most of the excess is related to higher chances of hospitalizations and outpatient unit visits not related directly to diabetes care. Diabetes care (diabetes nurse, dietitian, diabetes day centre, insulin and oral glucose agents, and blood sugar tests) constitutes smaller but still substantial part of the cost increase.

Conclusion: Thus, our results suggest that GDM on IADPSG criteria is associated with increased cost of maternity care and health care two to five years after the index pregnancy. This excess is not so much caused by the glucose tolerance treatment and monitoring routines as by more intensive use of hospital services, indicating worse health status of GDM women. Hence, this diagnosis objectively represents a serious condition altering health care cost around and beyond pregnancy. These findings provide information for further cost-effectiveness studies of the programs of GDM screening, treatment and monitoring in Irish setting.

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The effect of professional continuous glucose monitoring on glycaemic control and hypoglycaemia in insulin-requiring gestational diabetes mellitus

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Background and aims: Reducing hyperglycemia in gestational diabetes mellitus (GDM) improves pregnancy outcomes and reduces perinatal morbidity. Continuous Glucose Monitoring (CGM) is ideal for monitoring glucose levels where tight glycemic control without hypoglycaemia is required for a short period of time. While CGM is known to improve glycemic control/pregnancy outcomes in women with pre-gestational diabetes the same has

not been established in GDM. Aims: To determine if professional CGM improves glycemic control with less hypoglycaemia in insulin-requiring GDM. **Materials and methods:** In this prospective, open-label trial 24 women with insulin requiring GDM, gestation <28 weeks, were randomized to 2 groups. Group 1 (CGM) (n=11) underwent CGM using the iPro2 Enlite 6-day sensor at 28, 32 and 36 weeks gestation while Group 2 (control) did not. All patients performed 7 point fingerstick glucose profiles 3x/week, recorded hypoglycemic events (symptoms without glucose readings or fingerstick fasting glucose <3.5/nonfasting glucose <4.0 mmol/L) and were reviewed at 1-2 weeks intervals. In group 1, both CGM and fingerstick data were used to manage diabetes while women in group 2 were managed based on fingerstick data alone. HbA1c was measured at weeks 28, 33 and 37. Euglycemia on CGM was defined as 3.5-6.7 mmol/L.

Results: There were no significant differences in mean age, prepregnancy BMI, HbA1c and total insulin dose between groups at baseline. In the control group, mean HbA1c rose significantly from week 28 (5.31±0.52%) to 33 (5.42±0.56%) to 37 (5.6±0.63%), while HbA1c in the CGM group did not change significantly from week 28 (5.06±0.3%) to 33 (4.95±0.34%) and 37 (5.01±0.35%). HbA1c was significantly higher in controls compared with CGM at 33 and 37 weeks gestation. In the CGM group, 100% of patients at 37 weeks had HbA1c <5.6% compared to only 46.2% in controls (p=0.006). There was also a significant difference in mean change in HbA1c from 28 weeks to 37 weeks between the 2 groups (CGM -0.05%, control +0.29%, p=0.017). Mean time spent in euglycemia in the CGM group increased from the 1st to the 3rd CGMS at week 28 and 36 respectively (79.18±10.01% to 85.00±7.69%, p=NS) and mean time spent in hyperglycemia decreased (18.82±10.73% to 11.55±7.07%, p=NS). There was however an increase in mean time spent in hypoglycemia (2.00±2.83% to 3.45±3.78%, p=NS). 63.6% of patients in the CGM group (7/11) showed increased frequency of hypoglycemia from 28 weeks to 37 weeks compared to 30.8% (4/13) in the control group. The CGM group showed a significant increase in mean total insulin dose at 37 weeks compared to controls (52.91±27.92 vs 33.92±14.98 units, p<0.05). Mean birth weight in the CGM group was lower compared to controls (2851.8±323.3 vs 3065.8±464.0 g, p=NS).

Conclusion: Glycemic control in the women who underwent CGM was significantly better compared to women who received usual antenatal care based on fingerstick glucose alone. Use of CGM however, was associated with an increase in hypoglycemic events explained perhaps by the concomitant increase in total insulin received as a result of greater awareness of hyperglycemia by the managing team.

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Gestational diabetes mellitus: the first prospective randomised study of metformin-glyburide vs insulin

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Background and aims: Oral hypoglycemic agents (metformin - Met - and glyburide - Gly) have been used separately to treat gestational diabetes mellitus (GDM), with various failure rates imposing insulin therapy. Because GDM is mostly the consequence of insulin resistance and beta-cell deficiency, we reasoned that a combination of both drugs (Met-Gly) might efficiently treat women with GDM and may decrease the failure rate. For ethical and safety considerations, only half-maximal dosages were prescribed as per Health Canada suggestions. Here, we assessed maternal glycemic control (GC) and neonatal issues.

Materials and methods: We performed a randomized controlled study comparing a Met-Gly combination of half-maximal doses (Met 1250 mg/day, Gly 10 mg/day, with an incremental increase in dosages) to intensive insulin therapy (aspart/lispro and N/NPH) in women with GDM who did not achieve Canadian Diabetes Association treatment goals under diet (±exercise as indicated) therapy (capillary fasting glucose value < 5.3 mmol/L and 2-hr postprandial glucose value < 6.7 mmol/L). GC was defined as the 2-week mean of capillary glucose values (fasting and 2 hours after each 3 meals) measured daily (mean of 14 values for each recording time point).

Results: Treatments started at 29.3±3.8/30.1±3.1 weeks of gestation, respectively, in the Met-Gly (n=35) and the insulin (n=33) groups, while there was no difference between groups in age (31.1±4.7/30.7±4.4 yrs), weight

(85.3±17.5/85.3±22.9 kg), BMI (32.0±5.4/32.2±7.2 kg/m²) and GC recorded during the previous 2 weeks: fasting glucose value was 5.3±0.7/5.3±0.6; postprandial glucose values were: 6.3±0.8/6.3±0.7, 6.6±0.8/6.4±0.6 and 6.8±0.8/6.8±0.9 mmol/L, respectively post breakfast, lunch and supper. At delivery, in the Met-Gly group, 8 women were taking Met only (844±265 mg daily), 14 were taking Met (1179±153 mg) and Gly (3.9±1.9 mg), 10 were on Met-Gly+insulin (1333±250 mg, 8.6±2.2 mg and 12.7±9.9 units) and 3 were on insulin alone. In the 13 women taking insulin (37%), injections were started 4.2±2.1 weeks after initiation of Met-Gly treatment. There was no difference in insulin doses between women taking insulin alone in the Met-Gly group and women in the insulin group: breakfast: 7.0±4.2/11.3±9.0, lunch: 8.5±4.9/9.6±8.1, supper: 11.0±4.2/10.3±6.8, bedtime: 11.0±7.1/18.7±15.1 units. During the 2 weeks prior to delivery, no difference was observed in GC between groups (fasting: 4.7±0.3/4.8±0.3; postprandial 5.8±0.4/5.9±0.5; 5.8±0.5/5.9±0.5; 6.0±0.5/6.1±0.5 mmol/L). At delivery, we found no difference in the number of caesarean sections (9/8), neonatal birth weight (3360±389/3227±570 g), gestational age (38.7±1.1/38.4±1.5 weeks) or neonatal hypoglycemia (21/15).

Conclusion: Our data suggest that a majority of women with GDM could benefit from hypoglycemic agents: a combination of mild doses of Met-Gly successfully controlled GC in >60% women with GDM, with neonatal outcomes that are comparable to those of neonates born to women with GDM on insulin therapy.

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Metformin in gestational diabetes and fasting glucose as a predictor of treatment failure

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Background and aims: Gestational Diabetes (GDM) is common and affects up to 18% of pregnancy. Proper glycaemic control is the key to the reduction of GDM-related maternal and neonatal morbidity and mortality. The current guidelines suggest insulin or metformin only after dietary failure. Almost half of metformin-treated GDM need supplementary insulin. Identifying the characteristics of women who fail metformin may help to define optimal therapeutic strategy in GDM.

Materials and methods: This was a historical cohort of GDM in a District General Hospital, UK between 2009 and 2012. GDM was diagnosed by 75 g OGTT test between 24–28 weeks of gestation with fasting level ≥6.1 mmol/L and/or 2-hour post-prandial (PP) level ≥7.8 mmol/L. Treatment targets were: pre-meals 4–5.5 and 1-hour post meals <7.8 mmol/L. The treatment was escalated if 2–3 readings per week were above target. Daily metformin dose was 500–2000 mg. Non-parametric tests were applied appropriately to compare the treatment groups. Logistic regression and receiver operator curve (ROC) were performed to identify the predictors of metformin failure.

Results: Of the 299 being diagnosed and treated, complete treatment data were available in 228. Out of 228, 31(14%) were diet(D) alone, 141(62%) were on metformin(M) and 46(15%) on insulin(I) until delivery. 10 stopped metformin for gastrointestinal intolerance and were excluded from analysis. Compared to D group, despite older age(29 vs 32 years, p<0.05), greater BMI(27.45 vs 31.5, p<0.01), higher fasting(4.5 vs 4.9 mmol/L, p<0.01) & PP(8.1 vs 8.5 mmol/L, p<0.05) and earlier gestational age(GA) at OGTT (30⁺³ vs 26⁺⁴ weeks, p<0.001) in M group, the outcomes(birth weight, Apgar scores at 1 and 5 minutes, caesarean section, shoulder dystocia and NICU admission rates) were similar, but borderline higher average HbA_{1c} (5.35 vs 5.5 %, p=0.05) and earlier GA at delivery (39 vs 38⁺² weeks, p<0.01) in M group. The maternal characters between M and I groups had no significant difference, and M group had lower average HbA_{1c} (5.7 vs 5.5%, p<0.05) and all other outcomes were similar. 57% (80/141) of the metformin-treated GDM needed insulin. Both metformin success(M alone) and failure(M+I) groups had similar post-treatment HbA_{1c} (5.45 vs 5.5%, p=0.38). The failure group had higher maternal age (30 vs 32 years, p<0.05) and fasting level at OGTT (4.6 vs 5.2 mmol/L, p<0.001) and lower GA at dietary failure (29⁺² vs 27⁺⁶ weeks, p<0.01). Metformin failure was predicted if fasting OGTT level >4.8 mmol/L (69% sensitivity, 63% specificity). If the fasting level of IADPSG (International Association of Diabetes and Pregnancy Study Groups) criteria (≥5.1 mmol/L) was used, the positive predictive value was 79%. Premature delivery (<37 weeks) were higher in the failure group (OR 12.6; 95% CI 1.6, 99.8).

Conclusion: Metformin can be a better alternative than insulin for optimal glycaemic control in GDM. As metformin can benefit greater than diet alone, metformin should be initiated early at the diagnosis of GDM, based on fasting glucose at diagnosis. It is also important to consider adding basal insulin at initiation of metformin or alternative treatment option for the GDM identified by IADPSG fasting criteria for optimal treatment strategy.

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Type 2 diabetes mellitus genetic risk variant T in TCF7L2 rs7903146 in women is associated with an increased probability of insulin therapy in gestational diabetes mellitus

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Background and aims: Single nucleotide polymorphisms (SNPs) within the transcription factor 7-like 2 (TCF7L2) gene are well known risk variants for type 2 diabetes mellitus (T2DM). The rs7903146 SNP is involved in glucose- and incretin-induced insulin secretion and in proinsulin conversion. The aim of this international multicentric analysis was to assess and compare the effect of the TCF7L2 rs7903146 T risk variant on the development and clinical course of gestational diabetes mellitus (GDM).

Materials and methods: We genotyped the C/T polymorphism of the TCF7L2 gene variant rs7903146 in 150 unrelated Caucasian women with a history of GDM and in 160 non-diabetic pregnant women as control. The enrolment took place in Germany and in Greece. The prevalence of non-risk CC and risk TT alleles in the two groups was compared. Moreover, in the GDM group we evaluated the impact of the T risk allele on clinical sequelae and outcomes, defined as need for therapy with insulin and macrosomy of the offspring.

Results: The TT variant was associated with increased risk of GDM (p=0.021). Women carrying the T risk allele mutation had a lower BMI (mean 24.86 kg/m² for TT vs 26.06 kg/m² for CC), and a higher probability of initiation of insulin therapy, both in homozygotes (p=0.02) and heterozygotes (p=0.001) for the mutation. No statistically significant effect of the T allele on the development of macrosomy was detected (p>0.05).

Conclusion: The TT genotype of TCF7L2 rs7903146 is associated with an increased risk of GDM in Caucasian women. This confirms the hypothesis of a common genetic background between GDM and T2DM. Moreover, the T allele is associated with the necessity of therapy with insulin in GDM, both in homozygotes as well as in heterozygotes, independently from BMI. These results suggest an independent effect of the T risk allele on the severity of GDM.

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Blood pressure, microalbuminuria and weight gain in women with gestational diabetes

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Background and aims: Women with prior gestational diabetes mellitus (GDM) are at increased risk of developing type 2 diabetes and associated vasculopathy. Moreover, more than 30% of the women have overweight or obesity. The aim of the present work was to study the relationship between overweight/obesity and weight gain during the pregnancy and the development of hypertension in women with gestational Diabetes. Moreover, we studied the urinary albumin excretion and its possible relationship with blood pressure levels and the glucose abnormalities in women with GDM.

Materials and methods: We prospectively studied 422 women with GDM. We evaluated: glucose control (HbA_{1c}/monthly, need of insulin therapy), BMI (before the pregnancy), Weight gain (according to IOM, 2009). Blood pressure was measured at each office visit. We defined hypertension when the confirmed blood pressure levels were SBP/DBP ≥140/90 mmHg or antihypertensive treatment. Microalbuminuria and glomerular filtration by MDRD were measured. We collected obstetric history, personal history of GDM and personal and family history of cardiovascular risk factors.

Results: The mean age was 33.45 ± 4.5 yrs.; 26,25% smoking, 15,6% history of dyslipemia, 30% had history of previous abortions, 62,34% of women had first-degree relatives with type 2 DM and 59,25% had family history of hy-

pertension. 43 of the women with GDM (10,2%) presented hypertension during the pregnancy. The time of diagnosed of hypertension was $27 \pm 4,2$ weeks. The prevalence of overweight /Obesity (estimated by BMI before pregnancy) was significant higher in the group of GDM women with hypertension (mean BMI= $28,86 \pm 4,5$ kg/m²) during the pregnancy as compared to the GDM women without hypertension (mean BMI= $28,86 \pm 4,5$ kg/m²), 72% in the group with hypertension vs. 23% in the GDM women without hypertension ($p < 0.005$). The weight gain during the pregnancy was higher in the group of GDM women with hypertension (28% of them had an increase in their weight higher than the IOM recommendations, 2009), by contrast, only 13% of GDM women without hypertension. We found a significant relationship between the levels of SBP/DBP and the levels of urinary albumin excretion ($r = 0.42$, $p < 0.05$). Greater percentage of GDM women with hypertension needed insulin therapy for the glucose control as compared with GDM without hypertension ($p < 0.05$).

Conclusion: Women with GDM, the overweight/obesity before pregnancy and the increase in the weight during the pregnancy are related with the development of hypertension.

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Hypertension in type 2 diabetic pregnancy: 24hr ambulatory blood pressure monitoring vs conventional office cuff measurement

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Background and aims: There are only few data on hypertension in type 2 diabetic pregnancy. Hypertension diagnosis is conventionally based on office conventional measurements. However, nowadays new technologies exist that give relevant additional information on blood pressure. Our primary aims were: 1) To determine the additional value of 24h-blood pressure monitoring (ABPM) in terms of hypertension prevalence and prediction of pregnancy outcome in comparison to conventional approach. 2) To characterize and model the blood pressure profiles of the type 2 diabetic women that developed hypertension during pregnancy.

Materials and methods: Retrospective study of 52 type 2 diabetic pregnant women, age 24–39yrs, diabetes duration of at least 1 year (on average 9.5yrs), and average pregestational BMI of 26.99 kg/m^2 were followed throughout entire pregnancy. Blood pressure (BP) was measured with both the conventional method and 24h-ABPM. According to the guidelines, hypertension (conventional BP levels $\geq 140/90 \text{ mmHg}$) was classified as chronic, gestational, or preeclampsia. The 24h-ABPM (cut-offs 24hr $\geq 130/80$, daytime 135/85, night-time $\geq 120/70 \text{ mmHg}$) defined white-coat, sustained and masked hypertension. Data were processed using the IBM program 'SPSS 20'. X², Fisher's and Kruskal-Wallis test were performed when appropriate. P-values $< 0,05$ were considered significant.

Results: The two methods classified different patients as hypertensive. Conventional method identified 13 (25%) women with hypertensive disorders (6 sustained + 7 white-coat hypertension). 24h-ABPM identified 17 women with hypertensive disorders (32.7%) (6 sustained + 11 masked hypertension). Six women were classified as hypertensive with both methods (sustained hypertension). Thus 24h-ABPM hypothetically spared 7 subjects (white-coat) from unnecessarily intensive therapy. In the third trimester, short-term variability of both daytime ($p < 0.034$) and 24h-measurements ($p < 0.001$) of hypertensive women were significantly higher than in normotensive women. First trimester 24h-ABPM curves of women who developed hypertension later in pregnancy were consistently higher compared to those who didn't develop hypertension. Hypertensive disorders did not alter maternal and fetal outcome (just one case with preeclampsia).

Conclusion: 24h-ABPM showed significantly more accuracy in diagnosing and predicting the onset of hypertension already since the first trimester in type 2 diabetic pregnancies. Moreover, it succeeded in identifying masked, sustained and white-coat hypertension over the conventional method, fundamentally improving the opportunity for appropriate treatment. We would strongly suggest to use 24h-ABPM in the screening for hypertension in high risk groups such as type 2 diabetic pregnancies.

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Gestational diabetes and obstetric cholestasis: a 10 year retrospective cohort review of outcomes and predictors

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Background and aims: Obstetric cholestasis (OC) is a gestational liver disorder of unclear aetiology, associated with increased perinatal morbidity and mortality. Recent reports have suggested a link between OC and gestational diabetes (GDM), which also carries increased perinatal risks. Only two series of patients have been published to date with little information on what may predict the development of GDM in OC patients, and no Australian data currently published. Our aims were to look at the diagnosis of GDM associated with OC, review the outcomes of cases associated with both, and to screen for predictors that may indicate patients to be at increased risk of developing GDM.

Materials and methods: A retrospective case review was conducted of all pregnancies associated with antenatally-diagnosed OC (diagnosed by serum bile acids (SBA) $\geq 10 \mu\text{mol/L}$ with associated pruritus) at two major hospitals providing 80% of public antenatal care in South Australia, Australia between 2001 and 2010. All files were reviewed for severity of OC (severe = SBA $\geq 40 \mu\text{mol/L}$), fetal and maternal complications, presence of GDM (defined by Australian guidelines current at the time of pregnancy), and additional, including demographic, information deemed relevant. As an initial analysis binary predictors were analysed against the presence of GDM using the chi-squared test, and then by multivariate analysis using logistic regression. $P < 0.05$ was considered significant.

Results: 346 women were identified as being diagnosed with OC during 390 individual pregnancies over the 10 year period; 47 pregnancies were also affected by GDM (14% of total pregnancies affected by OC + GDM); 6 women had diabetes prior to pregnancy, and were excluded from subsequent analysis. There were no fetal deaths in OC + GDM pregnancies, and 85% of deliveries occurred at or after 36 weeks in this group (vs. 86% in OC only pregnancies; $P = 0.8$). Univariate analysis for predictors of GDM was conducted using the variables listed in the table; the only variables that remained significant in the multivariate analysis were [normal] Body Mass Index (BMI) ($P = 0.002$) and not being on ursodeoxycholic acid ($P = 0.001$).

Conclusion: The data supports the finding of an increased rate of GDM in pregnancies complicated by OC, at almost double the local rate when compared with the general pregnant population. This is the largest identified cohort to date with both conditions, and is the first series to suggest that normal BMI and not being on ursodeoxycholic acid may decrease the risk of developing OC-associated GDM. Although it is reassuring that there were no deaths, there is still the potential for a complicated course, and further investigation with larger cohorts, and more variables as potential predictors should be investigated.

Binary Variable <i>*italicised outcomes indicate the modelled outcome</i>	Univariate analysis (P)	Multivariate analysis (odds ratio, 95% confidence interval, P)
Race (Caucasian vs. non-Caucasian)	0.23	
BMI (BMI $\leq 25 \text{ kg/m}^2$ vs. $> 25 \text{ kg/m}^2$)	0.001	OR=0.27, CI 95% 0.12, 0.61, $P = 0.002$
Smoker (No/Yes)	0.3	
Alcohol use during pregnancy (No/Yes)	0.2	
Type of OC (Mild vs. Severe)	0.03	OR=0.83, CI 95% 0.40, 1.75, $P = 0.63$
Prior OC (No/Yes)	0.2	
OC diagnosed prior to 30 weeks	0.6	
Primigravida/multigravida	0.5	
Use of ursodeoxycholic acid (No/Yes)	< 0.001	OR=0.19, CI 95% 0.07, 0.50, $P = 0.001$

PS 094 Pregnancy outcomes in gestational diabetes mellitus

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Improved outcome of women with gestational diabetes mellitus

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Background and aims: The number of women with gestational diabetes mellitus (GDM) during pregnancy is increasing around the world, with a 10–100% increase in some race/ethnicity groups. GDM affects 1–14% of all pregnant women and is more common in populations with high frequency of type 2 diabetes. Among known risk factors for GDM is higher age, overweight, prior GDM and/or family history of diabetes. There are complications for the mother and child associated with GDM, both during and after pregnancy. With better maternity care and glucose control during pregnancy we expect improvement in outcome. The aim of this study was to compare pregnancy outcome of women with GDM 2013 against women with GDM 1995–1998.

Materials and methods: Since 1995 in our region in Sweden, all pregnant women are tested with a 2-hour oral glucose tolerance test (OGTT) consisting of 75 g glucose in solution as a general screening for GDM in the 28th gestational week. The cut off value for GDM in Sweden is ≥ 10.0 mmol/l in capillary plasma glucose and is based on the European Association for the Study of Diabetes (EASD) recommendations. Women who receive a GDM diagnosis are transferred to our specialised maternity unit with a team of obstetrician, diabetologist, midwife and dietician. In 2013, 115 women received GDM diagnosis and as comparisons we choose all women who had GDM during 1995–1998 (n=115) in our region. The women's medical journals from their GDM pregnancy were studied retrospectively. Duplex pregnancies (n=2 2013, n=3 1995–1998) as well as infants born before 37 weeks of gestation (n=10 2013, n=11 1995–1998) and after 41 weeks of gestation (n=0 2013, n=3 1995–1998) were excluded from the analysis regarding the newborn.

Results: When comparing women who had GDM 2013 against women with GDM 1995–1998, there was no significant difference between the groups regarding age of the mother, ethnicity, family history of diabetes, gestational length, HbA1c, insulin treatment during pregnancy, height of the mother, length of the newborn and apgar score at 5 and 10 minutes of the newborn. However, first weight of the women during GDM pregnancy 2013 was significantly higher than the weight of women with GDM 1995–1998, 71 kg (43–119) (n=114) and 64 kg (43–133) (n=111; $p=0.007$) respectively. However, there was no significant difference in weight of the mother at delivery. For women with GDM 2013 weight at delivery was 80 kg (54–130) (n=49) and for women with GDM 1995–1998 weight at delivery was 78 kg (55–138) (n=77; $p=NS$). Birth weight of the child in GDM pregnancies 2013 was $3508 \text{ g} \pm 487$ (n=81) and in GDM pregnancies 1995–1998 $3741 \text{ g} \pm 589$ (n=94; $p=0.005$). The percent of caesarean delivery was 24% in the GDM pregnancies 2013 and 17% in the GDM pregnancies 1995–1998 ($p=0.04$).

Conclusion: Even though women with GDM 2013 weigh more when they start the pregnancy there is no difference in weight at delivery compared to women with GDM 1995–1998. This is also reflected on the newborn, that 2013 had significantly lower birth weight but with the same gestational length as 1995–1998. We believe that this is due to a more active and intense treatment of women with GDM during pregnancy together with higher frequency of caesarean delivery. Prevention of large infants is crucial to avoid complications during delivery.

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Gestational diabetes: perinatal outcomes compared to background population and pregestational diabetes

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Background and aims: Prevalence of gestational diabetes (GD) ranges between 5–14% and is associated with an increased risk of maternal and fetal complications. The aim of this study was to assess the prevalence of GD and its outcomes in our population and to compare perinatal results with those of the general population (GP) and pregestational diabetes (PD).

Materials and methods: All patients with GD (NDDG criteria) who delivered at our centre (reference for our region) between January 2011 and December 2012 were identified, and their records were reviewed and compared to those with pregestational diabetes and the background population. Statistical analysis was performed using Microsoft Excel and SPSS for Windows. For frequency comparisons between groups, chi-squared was used. A bilateral $p < 0.05$ was considered significant.

Results: A total of 11480 deliveries were registered, 874 of whom (7.6%) had GD and 123 (1%) PD (44.6% type 1). Women with GD were more frequently above 35 than the GP (43.7% vs 28.9% $p=0.004$), similar to those with PD (41%), more frequently overweight/obese ($\text{BMI} > 25 \text{ kg/m}^2$) than the GP (62.6% vs 42.9% $p < 0.05$), but similar to the PD (75% $p=0.15$) and more often multiparous than the GP (68.4% vs 40.2% $p < 0.05$) and PD (54.6% $p=0.04$). There was a trend towards more induction of labour (42.3% vs 31.7% $p=0.06$) and caesarean sections (21.2% vs 14.8% $p=0.07$) in GD than in the GP, but less caesarean sections than in the PD (43.9% $p < 0.05$) and no differences in gestational age at delivery. No significant differences were found in perinatal mortality when compared to the GP (5.7% vs 3.8% $p=0.32$) or the PD (8% $p=0.7$). Macrosomy ($>4 \text{ Kg}$) was more frequently seen in the GD than in the GP (10% vs 0.9% $p < 0.05$), but less than in the PD (26.7% $p < 0.05$). Shoulder dystocia occurred as often as in the GP (1% vs 0.73% $p=0.75$), but less than in the PD (6.5% $p < 0.05$). No differences were found in Apgar scores or pH at birth. Admission to the Neonatal Unit was more frequent in the GD than the GP (19.4% vs 10.2% $p=0.004$) and tended to be less than the PD (28.5% $p=0.08$).

Conclusion: The prevalence of GD in our population is in the lower range of what has been described in other populations. Women with GD are at higher risk (age, obesity) and have worse perinatal results than the general population, although better than women with PD.

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1102

Maternal and infant outcomes of women with milder degrees of GDM treated with diet only compared to women with normal glucose tolerance

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Background and aims: The prevalence of GDM has increased worldwide in part as a result of increasing obesity rates but also as a result of the implementation of the new evidence based IADPSG criteria. GDM results in increased health care costs and efforts to reduce these costs without increasing adverse effects to mother and child need to be considered. Thus the aim of this study was to ascertain if any subset of milder degrees of GDM treated with diet only have comparable outcomes to those with NGT and thus may be suitable for ongoing management in a less intensive setting in primary or community care.

Materials and methods: This observational retrospective cohort study carried out a secondary analysis of the ATLANTIC DIP dataset. Two groups of women one with diet treated GDM and one with NGT were extracted from an anonymised dataset and compared. The impact of BMI in addition to a diagnosis of GDM was also analysed. The following maternal (caesarean section, polyhydramnios, pre-eclampsia) and infant outcomes (LGA, admission to neonatal unit, hypoglycemia and congenital malformations) were examined. Pearson's Chi squared test was used to ascertain statistical differences.

between GDM and NGT. Logistic regression was used to model relationships between maternal and infant outcomes and BMI as a categorical variable (<25, 25–30, >30) in the two groups.

Results: 5806 women were identified, 887 with GDM and 4919 with NGT. Baseline characteristics were similar between the groups. Women with GDM had a higher risk of polyhydramnios (OR= 3.06; 95% CI 1.72–5.44) and C-section (OR=1.32; 95% CI 1.06–1.66) compared to women with NGT. A BMI >30 further increased the risk of C-section (OR =2.76 95% CI 2.20–3.46). C-section rate in women with BMI >30 was twice as common in GDM compared to NGT women (60.3% vs 31.6%, $P<0.05$). Rates of PET were not influenced by GDM status but were increased with BMI >30 (OR=2.21 95% CI 1.55–3.16). Infants of mothers with GDM had a higher risk of hypoglycemia (OR 6.39 95% CI 3.34–12.3) and congenital malformations (OR= 1.77 95% CI= 1.37–2.29) compared to infants of NGT mothers. BMI did not impact on this any further. Rates of LGA were greatest with BMI >30 (OR 1.76 95% CI 1.44–2.16). Neonates of GDM mothers were twice likely to be admitted to the neonatal unit compared to neonates of NGT mothers (18.5% vs 9.1%, $P<0.05$).

Conclusion: Milder forms of GDM requiring dietary intervention only are associated with a higher risk of adverse maternal and infant morbidities when compared to women with NGT. These adverse events increase further in those with a BMI >30. Thus these women need to be monitored and treated in the high intensity multi disciplinary hospital environment and are not suitable for ongoing less intensive care in the primary care or community setting. *Supported by: HRB*

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Analysis of pregnancy outcomes in gestational diabetes mellitus based on the new IADPSG criteria compared to the former criteria

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Background and aims: Results from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study led the International Association of Diabetes and Pregnancy Study Groups (IADPSG) to propose new diagnostic criteria for Gestational Diabetes Mellitus (GDM). The new IADPSG criteria have lower glucose concentration thresholds, with an increase in GDM prevalence as a consequence. In our institution, these criteria were implemented in September 2011. The aim of this study was to determine if the use of the new diagnostic criteria led to changes in the management and in the occurrence of pregnancy complications among women diagnosed with GDM compared to the old diagnostic criteria.

Materials and methods: We conducted a retrospective study of women with GDM followed at a single multidisciplinary university center. We included all consecutive women between July 2009 and December 2010 (group 1) diagnosed using the old criteria (O'Sullivan followed by a 100 g oral glucose tolerance test) and between January 2012 and April 2013 (group 2) using the revised criteria (IADPSG consensus, 75 g oral glucose tolerance test in the fasting state). All women were instructed to adapt their diet and record self-monitoring of blood glucose according to international guidelines. No oral antidiabetic drugs were used. Parameters assessed were: percentage of women requiring insulin therapy; and pregnancy complications: caesarean section, macrosomia (birth weight >90th percentile) and preeclampsia. Management guidelines have not been modified between the two periods.

Results: We included 286 women with GDM, 129 in group 1 and 157 in group 2. Mean age was similar between groups (34.0±5.4 (group 1) versus 33.0±5.2 (group 2) [years], $P=0.12$), as were BMI (25.2±5.3 versus 26.1±5.1 [kg/m²], $P=0.17$) and weight gain during pregnancy (13.3±5.7 versus 13.2±6.9 [kg], $P=0.94$). There was a trend - although not significant - towards a lower percentage of women requiring insulin therapy in group 2 (55.0% (group 1) versus 43.3% (group 2), $P=0.06$). However, we did not find a significant difference in the number of caesarean section (38.9% versus 43.1%, $P=0.55$), the number of babies born with macrosomia (37.6% versus 33.0%, $P=0.74$) and the occurrence of preeclampsia (1.6% versus 1.3%, $P=1.0$).

Conclusion: Our study indicates that, when using the new IADPSG criteria, we include women with a less severe GDM, as less women required insulin in addition to dietary advice. There was no significant difference in caesarean section, macrosomia and preeclampsia.

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Excessive weight gain and pregnancy outcomes in pregestational and gestational diabetes

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Background and aims: Women with diabetes mellitus (DM) during pregnancy are at higher risk of adverse maternal and neonatal outcomes. Excessive gestational weight gain (GWG) is a potential risk factor for these adverse results. In 2009 the Institute of Medicine (IOM) established recommendations for appropriate GWG in non-diabetic women. We try to examine if excessive GWG, using IOM recommendations, in pregnancies with pregestational diabetes mellitus (PGDM) or gestational diabetes (GDM) is associated with higher adverse pregnancy outcomes.

Materials and methods: We performed a retrospective study of 2773 singleton pregnancies in women with DM [(259 PGDM (176 type 1 DM, 83 type 2 DM), 2514 GDM)]. Maternal weight and body mass index (BMI) had been recorded pre-pregnancy and at the time of delivery. GWG was calculated and compared with IOM guidelines to assess if the upper limit per BMI category was breached. Examined maternal outcomes included pre-eclampsia, gestational hypertension and cesarean delivery. Fetal outcomes included large for gestational age (LGA), macrosomia and neonatal morbidity. Multivariate analyses were performed and odds ratio calculated using a logistic regression analysis adjusted with age, parity, ethnicity, pre-pregnancy BMI, and neonatal sex.

Results: 1. Women with PGDM: 46,2% demonstrated excessive GWG. In the excessive GWG group a higher percentage were overweight (60,2%) or obese (56,2%, $p<0,01$). There were no significant differences in GWG between type 1 and type 2 DM. Excessive GWG was associated with higher odds for LGA (OR 2,5 , CI 1,4-4,7 $p<0,01$), macrosomia (OR 6,4 , CI 2,2-20 $p<0,01$) and cesarean delivery (OR 2,1, CI 1,1-3,9 $p<0,05$). 2. Women with GDM: 18,3% demonstrated excessive GWG. A higher percentage in the group with excessive GWG were overweight (40,2%) or obese (35,2%, $p<0,01$). Insulinization was more frequent in the excessive GWG group (43,0% vs 52,6%, $p<0,01$). Excessive GWG was associated with higher odds for LGA (OR 2,3 , CI 1,7-3,1 $p<0,01$), macrosomia (OR 2,2 , CI 1,5-3,3 $p<0,01$) and cesarean delivery (OR 1,6, CI 1,3-2,0 $p<0,05$).

Conclusion: Excessive GWG confers an additional risk for LGA, macrosomia and cesarean delivery of both GDM and PGDM. Multidisciplinary lifestyle intervention programs must be implemented to achieve weight control in all women with diabetes during pregnancy.

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Mental health in early pregnancy is associated with pregnancy outcome in women with pregestational diabetes

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Background and aims: This study aims to explore the role of early pregnancy locus of control, presence of anxiety and depression, and health-related quality of life for pregnancy outcomes in women with pregestational diabetes.

Materials and methods: A cohort of 148 pregnant women with diabetes (118 type 1 diabetes and 30 type 2 diabetes), originating from a clinical trial cohort from 2009 to 2011, completed three internationally validated questionnaires; Multidimensional Health Locus of Control, Hospital Anxiety and Depression Scale and 36-Item Short-Form Health Survey - SF 36 at 8 weeks of gestation. Selected pregnancy outcomes were preterm delivery (<37 weeks) and neonatal overweight (large for gestational age, birth weight >90th percentile). Differences between groups were analysed by unpaired t-test. Three women giving birth to a small for gestational age infant were excluded from the analyses of the large for gestational age group vs. appropriate for gestational age group.

Results: Women with preterm delivery (n=28) had worse quality of life scores for the SF-36 Mental Component Summary (mean 42.8 (SD 13.1) vs. 48.8 (9.7), $p=0.03$), SF-36 Emotional Role Limitations (58.3 (38.1) vs. 82.9 (31.3), $p=0.0005$) and SF-36 Mental Health (67.7 (20.4) vs. 75.2 (15.8), $p=0.04$) at 8 weeks of gestation, compared to women delivering at term. Both women delivering preterm and women delivering at term had high levels of internal locus of control (30.4 (5.5) vs. 29.8 (4.9), maximum score 36, $p=0.57$), and

comparable lower levels of locus of control attributed to chance, doctors and other people. The level of anxiety and depression was generally low without any differences between the groups. As compared to women with appropriate for gestational age infants (n=86), women with large for gestational age infants (n=59) had similar scores for locus of control, anxiety and depression, and health-related quality of life. High levels of internal locus of control were seen both in women with large for gestational age and appropriate for gestational age infants (30.0 (4.5) vs. 29.8 (5.3), $p=0.85$).

Conclusion: Women with pregestational diabetes who delivered preterm were characterised by worse mental quality of life in early pregnancy.

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Pregnancy outcome in depressive and GDM subjects in Bangladesh: a hospital based comparative study

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Background and aims: In Bangladesh the prevalence rate of depression in adult population is only 4.6%. Gestational Diabetes Mellitus (GDM) is also alarming here but the actual prevalence rate of both diseases in pregnancy is still lacking, though they are common and result in serious consequences for mother and foetus. To our knowledge there has been little research addressing the association between them in pregnancy and the outcome. These data are deficient even in South Asia let alone Bangladesh. This study was the corollary part of one study (which tried to find out prevalence of depression and associated factors, among GDM subjects and to compare with Non GDM) to reveal the outcome of pregnancy with depression and GDM and compared.

Materials and methods: A total of 748 pregnant women participated in the study. They were followed up from their 1st visit (not included after 13th week) to 1st week after delivery for at least 3 checkups. Depressive symptoms was scored following MADRS scale (0-12=not, 13-19=mildly, 20-34 moderately, 35-60=severely - depressed). Blood glucose was measured on every visit following WHO criteria; GDM was diagnosed within 24th to 28th weeks. Delivery procedure, Birth weight and APGAR score at 1st and 5th minute were assessed for the neonate.

Results: Prevalence of depression among pregnant women was 12.69%. The rate was higher in GDM (n 366) subjects (21.73%) with mean age 28.34 years than NGDM (n 382) subjects (7.73%) with mean age 27.17 years. Over all mean depressive score was higher at 3 stages in GDM group. Rate of caesarean section, number of live birth, and birth weight was higher but APGAR score at 2 stages were lower in GDM group. Mean age, Parity and birth weight of baby was higher but mean education years, mean APGAR scores of babies at both time period was lower in depressed groups (all 3 stages). Pregnant who were depressed specially in last trimester seem to have more rate of CS. Though gestational age at delivery did not vary much among the depressed and non depressed group. Chi square test and 't tests' proved there was significant association with depressive scores with gestational age and birth weight. Physical Exercise and Weight gain rate is under analysis

Conclusion: The important finding of this study was the elevated prevalence rate of depression in pregnancy which was greater than assumed. And pregnancy outcomes are strongly associated with GDM and Depression. Developing countries do not focus much on mental health but it is becoming ultimate necessity for future.

	NGDM	GDM	24 to 28 week		28 week before delivery		Within 1 week after delivery	
			Not depressed	Depressed	Not depressed	Depressed	Not depressed	Depressed
Mean Age Years	27.17±3.50	28.34±3.34	27.82±3.86	26.34±1.17	27.82±4.8	26.34±4.4	27.82±4.26	26.34±1.36
Mean Education year	12.8±0.22	11.82±1.14	12.86±0.8	12.25±0.2	12.82±0.8	11.21±0.3	12.86±0.85	11.56±0.27
Mean parity	1.76±0.36	2.25±1.8	1.87±1.54	2.16±1.16	1.86±1.56	2.25±1.2	1.87±1.56	2.46±1.17
Severest week at delivery	36.71±1.84	37.47±1.8	37.84±1.8	37.22±2.2	37.82±1.84	37.22±2.2	37.82±1.86	37.46±2.87
mode of delivery CS	60.9%	64.9%	68.8	66.3	68.7	64.2	70.8	66.8
Birth weight	3.54±0.4	3.4±0.4	3.2±0.4	3.3±0.3	3.35±0.46	3.47±0.37	3.35±0.46	3.37±0.4
APGAR 1 at 1st minute	7.82±1.2	7.75±1.3	8.8±1.2	8.46±1.3	8.85±1.26	8.35±1.2	8.85±1.26	8.65±1.25
APGAR 2 at 5th minute	7.25±1.1	6.45±1.1	7.8±1.2	7.3±1.2	7.85±1.26	7.05±1.16	7.85±1.22	7.46±1.27

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Atlantic DIP: diabetes in pregnancy is the most significant risk factor for caesarean delivery

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Background and aims: Diabetes mellitus (DM) is a common medical disorder of pregnancy. Caesarean delivery (CD) is associated with increased cost and maternal morbidity. Our aim was to determine the influence of DM on risk of CD in our cohort of patients enrolled in the Atlantic Diabetes in Pregnancy (DIP) study.

Materials and methods: Women with singleton pregnancies of 23 weeks or more estimated gestational age were identified and 6526 unique deliveries included. This included 4688 (71.8%) pregnancies with normal maternal glucose tolerance (NGT), 282 (4.3%) with pregestational diabetes mellitus type 1 and type 2 (PGDM) and 1556 (23.9%) with gestational diabetes mellitus (GDM). GDM was defined according to IADPSG criteria. Backwards stepwise logistic regression was utilised to evaluate the independent influence of selected variables on CD risk including diabetes mellitus (DM), maternal smoking and obesity, nulliparity, hypertension, ethnicity and macrosomia.

Results: In total, 1968 (30.16%) pregnancies resulted in CD. In the setting of NGT pregnancies there were 1196 (25.51%) with CD, 181 (64.18%) with PGDM and 591 (37.98%) with GDM ($p<0.001$). Evaluating the group as a whole using regression models, the presence of DM (either pregestational or gestational) significantly increased the odds for CD (aOR 1.73, $p<0.001$, 95% CI 1.51-1.99). Maternal obesity was also noted to be a significant risk factor (aOR 1.72, $p<0.001$, CI 1.509-1.974). Other identified risks for CD in our cohort include foetal macrosomia (aOR 1.61, $p=0.003$, CI 1.18-2.2), hypertension (aOR 1.66, $p<0.001$, CI 1.38-2.01), nulliparity (aOR 1.45, $p<0.001$, CI 1.27-1.65) and increasing maternal age (aOR 1.05, $p<0.001$, CI 1.04-1.06). When GDM was evaluated as an independent risk factor, it was also found to increase the odds for CD (aOR 1.36, $p<0.001$, CI 1.18-1.58) and analysing this subgroup of women (n=1556), persistent risk factors for CD include obesity (aOR 1.99, $p<0.001$, CI 1.57-2.52) and maternal age (aOR 1.04, $p=0.002$, CI 1.01-1.07). In the setting of PGDM (n=181), this underlying diagnosis greatly increases the odds of CD (aOR 4.95, $p<0.001$, CI 3.75-6.52) and was the only independent risk identified within this group of women.

Conclusion: Despite significant advances in the management of patients with diabetes in pregnancy, DM remains the most significant, independent risk factor for the adverse outcome of CD. Future research should focus on methods of reducing this risk.

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PS 095 Postpartum outcomes

1108

Evaluating haemoglobin A_{1c} and metabolic syndrome versus oral glucose tolerance test in postpartum diabetes screening

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Background and aims: Gestational diabetes mellitus (GDM) increases the risk of type 2 diabetes (DM-2). Guidelines of clinical practice recommend postpartum screening with oral glucose tolerance test (TTOG). Compliance rates of women performing this test are low because this is an uncomfortable test. We investigated the reliability of other postpartum screening test in order to increase the compliance of women in postpartum.

Materials and methods: We evaluated 419 women with a history of GDM in postpartum screening using hemoglobin A_{1c} (A1C), HDL cholesterol, triglycerides, blood pressure and waist circumference and compared them with TTOG one year after delivery. We classified these patients into normal or pathological carbohydrate metabolism according to TTOG, A1C (following ADA criteria) and diagnostic criteria for the metabolic syndrome (MS) attending to ATPIII Expert Panel.

Results: Based on the TTOG 257 women (61,3%) had normal carbohydrate metabolism and 162 women (38,7%) had a pathological status: 69 (16,5%) impaired fasting glucose (IFG), 43 (10,3%) impaired glucose tolerance (IGT), 30 (7,2%) IFG+IGT and 14 (4,7%) had diabetes. According to the A1C criteria, 331 (77,9%) had normal carbohydrate metabolism and 84 (19,8%) had a pathological status: 82 (19,3%) prediabetes and 2 (0,5%) had diabetes. Attending to MS, 341 (80,2%) were normal and 64 (15,1%) had at least 3 diagnostic criteria for MS.

Conclusion: A1c and MS diagnostic criteria underestimate carbohydrate metabolism reassessment in women with a history of GDM compared to the gold standard TTOG.

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The role of HbA_{1c} for detection of diabetes and abnormal glucose tolerance after pregnancy with gestational diabetes mellitus

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Background and aims: Recently, HbA_{1c} has been proposed as a diagnostic tool to identify individuals with undiagnosed diabetes, or who are at risk of diabetes. Women with gestational diabetes mellitus (GDM) represent a high risk group for type 2 diabetes development. According to Swedish national guidelines life style intervention and follow-up of these women after pregnancy have high priority, but it is not made clear by which measures these women should be followed. The aim of the present study was to compare the performance of HbA_{1c} with established glucose criteria during an oral glucose tolerance test (OGTT) and to assess HbA_{1c} as a screening test for undiagnosed diabetes and pre-diabetes after pregnancy with GDM.

Materials and methods: Glucose homeostasis was reevaluated 1-5 years after delivery in 140 women with prior GDM by means of OGTTs and simultaneous HbA_{1c} measurements. Glucose tolerance was defined according to World Health Organization criteria. HbA_{1c} \geq 48 mmol/mol was used for diabetes diagnosis and HbA_{1c} 39-47 mmol/mol to define high risk women (pre-diabetes).

Results: Mean \pm SD for age and body mass index of included women were 35.4 ± 5.6 years and 26.6 ± 2.3 kg/m², respectively. A median (interquartile range) of 26 (21-60) months had elapsed since their GDM pregnancy. Based on the OGTT 62 women (44%) had normal glucose tolerance, 50 (36%) pre-diabetes, and 28 (20%) diabetes, as compared to 114 (81%), 21 (15%) and 5 (4%), respectively, using HbA_{1c} criteria. The agreement between diagnoses resulting from HbA_{1c} and OGTT criteria was estimated by constructing cross tables and calculation of the κ coefficient (κ). The consistency in classifying diabetes versus non-diabetes was 82% (115/140) and κ 0.194, indicating poor agreement. The corresponding figures for the classification of any degree of abnormal glucose tolerance were 59% (82/140), κ 0.227. Combining HbA_{1c} with fasting glucose criteria improved the agreement to fair (κ 0.596), but was no better than using the fasting glucose test alone (κ 0.599). The area under the receiver operating characteristic curve for HbA_{1c} and fasting glucose to detect any degree of abnormal glucose tolerance was 0.708 and 0.834, respec-

tively. Diagnostic accuracy was assessed using sensitivity, specificity, positive predicted value (PPV) and negative predictive value (NPV). Proposed cut points of HbA_{1c} had low sensitivity (30%) and modest NPV (52%) to detect any degree of abnormal glucose tolerance, whereas specificity and PPV were high. The combined use of HbA_{1c} and fasting glucose criteria improved the performance (sensitivity 67%, specificity 95%, PPV 95%, NPV 69%), but was similar to the use of the fasting glucose test alone. The latter identified 63% (49/78) of the women with pre-diabetes or diabetes in the study cohort. However, combined with a lower cut point of HbA_{1c} (\geq 31 mmol/mol), an additional 59 women were identified, among whom 36% had pre-diabetes or diabetes based on post glucose load hyperglycemia.

Conclusion: Proposed thresholds of HbA_{1c} had low diagnostic sensitivity. Combined with a fasting glucose test the performance was no better than using a fasting glucose test alone. Considering that early detection of pre-diabetes is of outmost importance in these women to prevent the development of diabetes, combining a fasting glucose test with a lower cut point of HbA_{1c} may be an alternate approach to select women for an OGTT to identify those with isolated post glucose load hyperglycemia.

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Glucose intolerance after a recent history of gestational diabetes

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Background and aims: The prevalence and the risk factors for glucose intolerance in early postpartum remain unclear in different populations. Our aim was to evaluate the uptake of our current screening strategy postpartum, the prevalence and the risk factors for glucose intolerance in women with a recent history of gestational diabetes (GDM).

Materials and methods: Retrospective analysis of the medical files of our university hospital from 01-01-2010 till 31-12-2013 of women with a recent history of GDM diagnosed with the Carpenter & Coustan criteria. Since 2010 all women with a history of GDM are advised to undergo a 75g oral glucose tolerance test (OGTT) between 6-24 weeks postpartum. Indices of insulin sensitivity (the Matsuda index and the reciprocal of the homeostasis model assessment of insulin resistance, 1/HOMA-IR) and an index of beta-cell function, the Insulin Secretion-Sensitivity Index-2 (ISSI-2) were calculated based on the OGTT during pregnancy and postpartum. Multivariable logistic regression was used to adjust for confounders.

Results: Over a 4 year period, 231 women were identified with a recent history of GDM. Of all women, 21.4% (46) did not attend the scheduled postpartum OGTT. Compared to women who received an OGTT postpartum, women who did not attend the postpartum OGTT smoked more often before pregnancy (10.9% vs 2.4%, $p=0.021$), had more often a previous history of GDM (21.7% vs 10.7%, $p=0.042$), less often breastfed (56.5% vs 75.7%, $p=0.011$) and had a lower insulin sensitivity based on the OGTT during pregnancy [the Matsuda index 2.2 (1.5-3.1) vs 2.8 (1.9-3.8), $p=0.033$ and 1/HOMA-IR 0.015 (0.010-0.023) vs 0.020 (0.012-0.032), $p=0.030$]. Of all women (169) who received an OGTT postpartum, 40.8% (69) had prediabetes (14.9% impaired fasting glucose, 32.5% impaired glucose tolerance and 7.1% both impaired fasting and impaired glucose tolerance) and 5.3% (9) had overt diabetes. Compared to women with a normal OGTT postpartum, women with prediabetes or diabetes were more often overweight (39.7% vs 25.3%, $p=0.009$) or obese at first prenatal visit (27.4% vs 19.5%, $p=0.037$), were more often multiparous (44.0% vs 26.6%, $p=0.028$), had a higher glucose challenge test [168.0mg/dl (153.0-190.0) vs 159.0mg/dl (149.5-175.5), $p=0.007$], had an earlier diagnosis of GDM [gestational weeks 26.0 (25.0-28.0) vs 27.0 (25.0-29.0), $p=0.030$], had an higher median fasting [94.5mg/dl (84.2-101.7) vs 88.0mg/dl (81.0-99.0), $p=0.006$] and 2 hour glucose value during pregnancy [175.0mg/dl (162.0-198.0) vs 168.0mg/dl (158.0-181.0), $p=0.003$] and were more often treated with basal-bolus insulin injections (52.0% vs 17.4%, $p=0.032$). Women with glucose intolerance postpartum also had a lower beta-cell function and lower insulin sensitivity, remaining significant after adjustment for age, BMI, ethnicity, breastfeeding, contraception, multiparity and corticoid treatment [ISSI-2 in pregnancy 1.5 ± 0.5 vs 1.7 ± 0.4 , $p=0.029$; ISSI-2 postpartum 1.5 (1.2-1.9) vs 2.2 (1.8-2.6), $p=0.020$; Matsuda index postpartum 3.8 (2.6-6.2) vs 6.0 (4.3-8.8), $p=0.021$].

Conclusion: Glucose intolerance is frequent in early postpartum and these women have a lower beta-cell function and lower insulin sensitivity. One fifth

of women did not attend the scheduled OGTT postpartum and these women have an adverse risk profile compared to women who received the OGTT postpartum.

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The risk of future glucose intolerance in women with history of gestational diabetes

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Background and aims: In women with gestational diabetes mellitus (GDM) abnormal glucose metabolism normalizes soon after delivery. However, the history of GDM predisposes to carbohydrate intolerance in the future. The aim of this study was to evaluate risk factors predicting to future development of diabetes mellitus (DM) in women with a history of GDM.

Materials and methods: 221 women with history of GDM were enrolled into the study. A 75 g OGTT was performed within 3 months following delivery (n=181) or 11±2 years after delivery(n=40). Based on ADA criteria participants were classified into 3 groups as normal glucose tolerance; NGT (group 1), IGT and/ or IFG (group 2) and DM (group 3). We evaluated the relationship between status of carbohydrate metabolism after delivery and possible risk factors such as: age-at-gestation; BMI; family history of DM; poor obstetric history; gestational week, A1C and OGTT glucose levels at diagnosis of GDM; weight gain and insulin requirement during index pregnancy.

Results: As shown in the table below, 16.2 % of participants had any degree of glucose intolerance after delivery. Gestational age of women who progressed to prediabetes or diabetes was lower than the others. At diagnosis OGTT glucose levels were higher and family history and poor obstetric history was much more higher in group 2 and 3. Women who developed later carbohydrate intolerance had insulin requirement during pregnancy.

Conclusion: Age-at-gestation, having poor obstetric history, higher glucose levels (fasting, 1st, 2nd hr) at diagnosis of GDM and insulin requirement during pregnancy are crucial predictors of development of glucose intolerance in the future. Those women required careful and more frequent follow-up after delivery.

	Group 1(NGT)	Group 2(IGT+IFG)	Group 3DM	p*
n(%)	185 (83.7)	25(11.3)	11(4.9)	
Age-at-gestation (yrs)	33.4±5.2	32.8±6.9	27.2±5.3	0.007
BMI (kg/m ²)	29.7±4.6	30.6±3.0	29.8±3.9	0.295
Weight gain (kg)	10.2±4.7	9.7±3.9	8.4±5.0	0.569
Family history of DM	1.78±1.91	2.1±2.4	2.6±1.6	0.134
Poor obstetric history	0.09±0.35	0.25 ±0.68	0.82±1.8	0.041
A1C (%)	5.4±0.5	5.5±0.6	5.2±1.0	0.093
Insulin requirement (%)	44.5	56	72.7	0.042
At diagnosis glucose (mg/dl)				
Fasting	92.9±12.9	99.7±12.0	116.9±25.7	<0.001
1st h	195.4±28.4	203.4±14.9	257.1±41.7	<0.001
2nd h	163.2±36.3	185.8±38.9	245.8±52.5	<0.001
3rd h	121.3±35.4	126.6±42.0	167.0±60.6	0.093

*Kruskal Wallis Test

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Biomarkers of endothelial dysfunction in relation to impaired carbohydrate metabolism early after pregnancy with gestational diabetes mellitus

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Background and aims: The diagnosis of gestational diabetes mellitus (GDM) implicates an increased risk for the later development of type 2 diabetes. It was previously shown that metabolic reassessment early after delivery identifies females with particularly high risk. However, the relation to endothelial dysfunction, which might represent an early precursor of atherosclerosis and cardiovascular disease in females after pregnancy with GDM, is less well understood.

Materials and methods: 108 women with previous gestational diabetes (pGDM) and 40 controls were included 3-6 months after delivery and underwent specific metabolic assessments including a frequently sampled intravenous glucose tolerance test (FSIGT) and an oral glucose tolerance test (OGTT) to derive parameters of insulin sensitivity and β -cell dysfunction in addition to dynamics of glucose, insulin, C-peptide, proinsulin and amylin. The area under the curve was calculated by the trapezoidal rule including measurements of fasting, as well as 30', 60', 90' and 120' post load concentrations. OGTTs were repeated in females with pGDM over 10 years of follow-up to identify subjects with diabetes manifestation. Circulating ICAM-1 (intracellular-adhesion-molecule-1), VCAM-1 (vascular-cell-adhesion-molecule-1) and E-selectin, representing biomarkers of endothelial dysfunction were assessed at baseline and annually over five years after index pregnancy.

Results: Adhesion molecules and E-selectin were significantly related to insulin sensitivity estimated from the FSIGT (SI-FSIGT): ICAM-1: $r=-0.23$, $p=0.009$; VCAM-1: $r=-0.22$, $p=0.011$; E-selectin: $r=-0.21$, $p=0.018$. Fractional polynomial regression models revealed further associations between ICAM-1 and AUC-glucose ($p=0.003$), AUC-proinsulin ($p=0.005$) and AUC-amylin ($p=0.017$) as well as between E-selectin and AUC-glucose ($p=0.013$) and BMI ($p=0.004$) after adjustment for the degree of insulin resistance. Moreover, adhesion molecules remained significantly elevated in pGDM subjects as compared to the control group in a multivariable model including SI-FSIGT, age and BMI. 21% of females after pregnancy with GDM developed diabetes during the follow-up period. Longitudinal analysis revealed significantly higher ICAM-1 and E-selectin levels in subjects with progression to overt diabetes.

Conclusion: Biomarkers of endothelial dysfunction are related to impaired carbohydrate metabolism in subjects with recent history of GDM. This observation might indicate the later development of overt cardiovascular disorders in this specific risk collective. Follow-up examinations with clearly defined cardiovascular endpoints are recommended to perform an accurate risk stratification.

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1113

Non-alcoholic fatty liver disease is prevalent in non-diabetic women with previous gestational diabetes mellitus regardless of their glucose tolerance

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Background and aims: Gestational diabetes mellitus (GDM) increases the risk of developing type 2 diabetes later in life. Furthermore, type 2 diabetes and obesity are associated with non-alcoholic fatty liver disease (NAFLD).

NAFLD is defined as a condition with excessive fat accumulation in the liver and absence of significant alcohol consumption. We examined glucose tolerance and fat distribution in obese, non-diabetic women with prior GDM.

Materials and methods: Based on a 75g-OGTT, non-diabetic women with previous GDM were classified as having normal glucose tolerance (NGT) or prediabetes (impaired fasting glucose and/or impaired glucose tolerance). Insulin resistance (IR) was assessed by HOMA-IR. All women underwent an ultrasound scan of the liver to detect fat accumulation, blood sampling, and a dual energy X-ray absorptiometry (DXA) scan. The android-to-gynoid fat-ratio and total visceral fat mass were calculated based on the DXA scan. A questionnaire was used to evaluate alcohol consumption habits.

Results: Women with previous GDM were included ($n=70$; age: 38 ± 5 years (mean \pm SD); BMI: 31 ± 5 kg/m²; HbA_{1c}: 34 ± 4 mmol/mol ($5.4\pm 0.4\%$); HOMA-IR: 1.2 ± 0.6 ; duration since GDM: 5 ± 2 years). Twenty four women had NGT (fasting plasma glucose (FPG) 5.4 ± 0.3 mM; 2h-OGTT plasma glucose (PG): 6.7 ± 1.4 mM) and 46 women had prediabetes (FPG 5.5 ± 0.5 mM; 2h-OGTT PG: 9.2 ± 1.1 mM). NAFLD was diagnosed in 5 (21%) women with NGT and 15 (33%) with prediabetes). Glucose tolerance status (NGT or prediabetes) did not coincide with NAFLD ($p=0.39$). Multivariable logistic regression analyses showed that NAFLD was positively associated with HOMA-IR ($p=0.035$), BMI ($p=0.004$), C-peptide ($p=0.038$), mass of visceral fat ($p=0.004$), the android-to-gynoid fat-ratio ($p=0.027$), and plasma levels of alanine aminotransferase ($p=0.018$) and aspartate aminotransferase ($p=0.028$).

Conclusion: NAFLD is prevalent in non-diabetic women with previous GDM regardless of their glucose tolerance status, but is associated with HOMA-IR, high BMI, high C-peptide levels, android fat distribution, and elevated plasma liver enzymes.

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Incretin function in adult offspring of women with diabetes in pregnancy

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Background and aims: Fetal exposure to maternal diabetes is associated with increased risk of pre-diabetes and type 2 diabetes (T2DM) in the offspring. The pathogenesis of T2DM seems to involve dysfunction of the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), as well as hyperglucagonemia. Our aim was to investigate the levels of the incretin hormones GIP, GLP-1 and glucagon in adult offspring exposed to intrauterine hyperglycemia.

Materials and methods: A cohort of 587 Caucasian offspring, without known diabetes was followed up at the age of 18–27 years. We included two groups exposed to maternal diabetes in utero: offspring of women with gestational diabetes mellitus (O-GDM) or type 1 diabetes (O-T1DM). Two reference groups were included: offspring of women with risk factors for GDM, but normo-glycemia during pregnancy (O-NoGDM) and offspring from the background population (O-BP). The subjects underwent a 75-g oral glucose tolerance test (OGTT) with venous blood samples at 0, 30, 120 min.

Results: Significantly lower levels of GLP-1 in the fasting state was found in the 2 diabetes-exposed groups (O-GDM and O-T1DM) compared to O-BP ($p=0.032$ and 0.004 respectively). The levels of glucagon during OGTT (time=30 min.) showed a tendency towards higher level in O-GDM compared to the unexposed groups (O-NoGDM and O-BP). No association between levels of GIP and exposure status was found.

Conclusion: Reduced levels of GLP-1 in the fasting state and increased levels of glucagon during OGTT may contribute to the increased risk of glucose intolerance among adult offspring born to women with diabetes during pregnancy.

PS 096 Pregnancy in type 1 diabetes

1115

Hyperglycaemia remains the major risk factor of major foetal complications in pregnant women with type 1 diabetes and good glycaemic control

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Background and aims: In women with type 1 diabetes pregnancy is associated with increased risk of adverse outcomes. The aim of this study was to ascertain the association of glycemic control, estimated with HbA_{1c}, with selected serious adverse pregnancy outcomes, such as preterm labor, congenital malformations low birth weight and stillbirths in a group of pregnant women with very good metabolic control.

Materials and methods: The study cohort comprised 510 women with type 1 diabetes. They received intensive diabetes management during the period of pregnancy in an academic outpatient reference center between 1998 and 2012. We excluded multiple pregnancies ($n=9$), miscarriages (before 20th week of gestation, $n=40$), cases with missing outcomes and lost to follow-up ($n=6$). Of the remaining 455, 183 women (40.2%) entered the intensive management program before conception (pregnancy planning). HbA_{1c} was measured in 3 pregnancy trimesters, in a laboratory implementing internal and external quality assurance protocols, with an assay (high performance liquid chromatography) that was adjusted to DCCT standard.

Results: The median HbA_{1c} in the 1st, 2nd and 3rd trimester was 6.5%, 5.7% and 5.6%, respectively. Only 155 (34.1%) women in the 1st trimester, 44 (9.7%) in 2nd and 39 (8.6%) in 3rd had HbA_{1c} 7.0% or higher. In spite of that, we observed 70 (15.4%) preterm labors (two between 20th and 26th week of gestation). There were 34 (7.5%) cases of low birth weight (below 2500 g), 10 of them occurred in term newborns. There were also 10 (2.2%) stillbirths and 28 (6.1%) congenital malformations in live births. Additional 4 cases of congenital malformations occurred in stillborn fetuses. The strongest predictor of preterm delivery was the 3rd trimester HbA_{1c}: relative odds of preterm delivery per 1% increase in HbA_{1c} was 1.46, $p=0.004$. First and 2nd trimester HbA_{1c} had weaker impact: odds ratios were 1.20 ($p=0.06$) and 1.35 ($p=0.01$), respectively (both per 1% increase in HbA_{1c}). Of note, 97% preterm labors took place after 26th week of gestation. Interestingly we did not observe significant relationship between low birth weight and HbA_{1c}, while the odds of this complication increased by 1.48 ($p=0.02$) per each additional 5 years of maternal age. The 1st trimester HbA_{1c} (but not 2nd or 3rd) was associated with the risk of stillbirths: relative odds per 1% increase of HbA_{1c} was 1.70, $p=0.005$. Similarly, 1st trimester HbA_{1c}, but not 2nd or 3rd, was associated with the risk of congenital malformations (relative odds per 1% increase of HbA_{1c} was 1.41, $p=0.005$). Although pregnancy planning resulted in 0.8% (6.8 vs. 6.0%) reduction of median HbA_{1c} in the 1st trimester and 0.3% (5.8 vs. 5.5%) in the 3rd, it was not sufficient to significantly lower the risk of adverse outcomes (odds ratio 0.80, $p=0.35$ for the composite outcome of preterm deliveries, stillbirths and malformations).

Conclusion: Despite very good glycemic control the risk of the examined adverse outcomes of pregnancy in women with type 1 diabetes remains high. In this study preterm labors were associated predominantly with the 3rd trimester HbA_{1c} but less with earlier glycemic exposure. Hyperglycemia during the first trimester of pregnancy, the period of embryonic organogenesis, was associated with increased risk of congenital malformations and stillbirths. These results emphasize the need for maintaining normoglycemia throughout the whole course of pregnancy.

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Fixed rate intravenous insulin infusion for euglycaemic diabetic ketoacidosis to a pregnant type 1 diabetic with good foetal outcome

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Background and aims: Diabetic Ketoacidosis (DKA) is a life threatening condition during pregnancy. It carries high mortality (up to 30%) for both infant and mother. Ketosis has been implicated in foetal distress and causes adverse neurological outcome. During pregnancy, euglycemic diabetic ketoacidosis requires prompt recognition and treatment. The current national guideline in UK recommends using a fixed dose of insulin infusion (0.1units/kg/hr),

titration of fluids to keep this infusion continuously running and continuation of long acting insulin during management of DKA. We report a case of euglycemic DKA in a pregnant Type 1 diabetic who received fixed rate insulin infusion with complete recovery.

Materials and methods: A 24 year old type 1 diabetic for 10 years attended the antenatal clinic in a District General Hospital for antenatal management of her 2nd pregnancy. Her antenatal period had been uneventful till 24 weeks, with a satisfactory diabetes control (HbA1c of 50 mmol/mol) on a basal bolus regimen. She presented to the antenatal clinic with history of nausea, recurrent vomiting, abdominal pain and headache for preceding 6 days. She had stopped taking her short acting insulin but continued with her basal insulin. Clinically she was dehydrated. Investigations showed plasma glucose 6.5 mmol/l and blood ketones 3.9. Venous blood gas showed acidity (PH) 7.21, bicarbonate 9 and lactate 0.9. Rest of the biochemical investigations were unremarkable. Cardiotocography for fetal monitoring was normal.

Results: A diagnosis of euglycemic diabetic ketoacidosis was confirmed, fixed dose of intravenous insulin with dextrose infusion was initiated as per Joint British Diabetes Societies (JBDS) guidelines. She was transferred to HDU for close monitoring. No precipitating factors for DKA was found. She made an excellent recovery and discharged home in 2 days. She delivered by caesarean section at term with good foetal outcome.

Conclusion: Euglycemic DKA is poorly understood in non-specialist practice, but it is important to recognize the condition early. Poor oral intake due to vomiting and nausea as well as pregnancy were the only contributing factors in our case. Fixed rate intravenous insulin has been part of the DKA pathway (JBDS Guidelines) only recently along with bedside measurement of metabolic changes. The rationales for this recommendation are: faster resolution of ketosis with higher insulin and glucose concentrations thereby rapid recovery with reduction in the length of stay, and significant delay in the normalisation of pH with a sliding scale protocol. Euglycemic DKA, although uncommon, still continues to be perceived in clinical practice. Type 1 diabetic patient presenting with nausea and vomiting and found to have a normal glucose may still have life-threatening ketoacidosis, and an evaluation of their acid/base status is still revealed. Identification of such presentation timely is crucial and also the need for early fixed rate intravenous insulin infusion along with dextrose which will prevent significant morbidity for both the mother and the foetus like in our case.

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Higher gestational weight gain is associated with increasing offspring birth weight independent of maternal glycaemic control in women with type 1 diabetes

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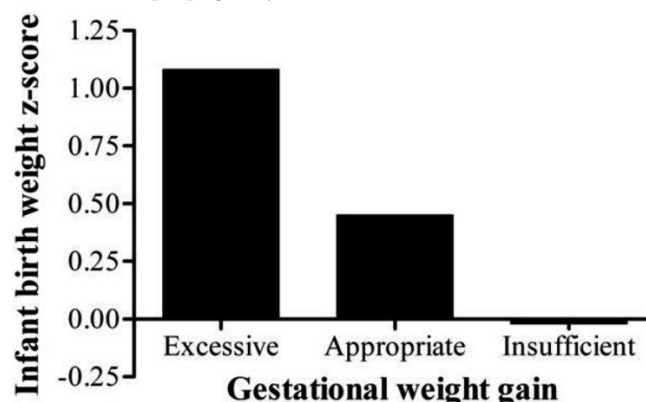
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Background and aims: To evaluate the association between gestational weight gain and offspring birth weight in singleton term pregnancies of women with type 1 diabetes.

Materials and methods: One-hundred-and-fifteen consecutive women referred <14 weeks were retrospectively classified as underweight (pre-pregnancy BMI <18.5 kg/m², n=1), normal weight (18.5–25.0, n=65), overweight (25.0–29.9, n=39) or obese (≥30.0, n=10). Gestational weight gain was categorized as excessive, appropriate or insufficient according to the Institute of Medicine recommendations for each BMI class.

Results: HbA1c at 8 and 36 weeks was comparable between women with excessive (n=62), appropriate (n=37) and insufficient (n=16) gestational weight gain (median 6.7 (range 5.6–8.4) vs. 6.5 (5.4–8.3) vs. 6.6 (5.6–8.3) % (50 (38–68) vs. 48 (36–67) vs. 49 (38–67) mmol/mol), p=0.78, and 6.0 (5.1–6.9) vs. 6.0 (4.7–7.1) vs. 6.3 (5.1–7.0) % (42 (32–52) vs. 42 (28–54) vs. 45 (32–53) mmol/mol), p=0.40, respectively) and pre-pregnancy BMI was 25 (18–41) vs. 24 (18–31) vs. 23 (20–30) kg/m² (p=0.05). Offspring birth weight and birth weight z-score decreased across the groups (3,681 (2,374–4,500) vs. 3,395 (2,910–4,322) vs. 3,295 (2,766–4,340) g, p=0.02, and 1.08 (–1.90–3.25) vs. 0.45 (–0.83–3.18) vs. –0.02 (–1.51–2.96), p=0.009). In a multiple linear regression analysis, gestational weight gain (kg) was positively associated with offspring birth weight (g) (β=19, p=0.02) and birth weight z-score (β=0.06, p=0.008), when adjusted for pre-pregnancy BMI, HbA1c at 36 weeks, smoking, parity and ethnicity.

Conclusion: Higher gestational weight gain in women with type 1 diabetes was associated with increasing offspring birth weight independent of glycaemic control and pre-pregnancy BMI.



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Abnormal renin-angiotensin system in early pregnancy in a prospective cohort of women with type 1 diabetes

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Background and aims: The renin-angiotensin system plays an important role in regulation of blood pressure, and abnormalities predispose to cardiovascular disease. Preeclampsia (PE), a hypertensive disorder of pregnancy, is ~4 fold more prevalent in women with diabetes, but underlying mechanism is not fully understood. In this prospective study of type 1 diabetic (T1DM) women, we aimed to determine whether plasma levels of angiotensin-converting enzyme (ACE), angiotensinogen, and prorenin were altered early in pregnancy in women with subsequent PE, and whether they could serve as early markers for PE.

Materials and methods: Maternal plasma ACE, angiotensinogen and prorenin were measured longitudinally by ELISA at approximately 12, 22, and 32 weeks of pregnancy in 23 women with T1DM who subsequently developed PE (DMPE+), 24 diabetic women who did not have PE (DMPE-), and 20 non-diabetic normotensive women (DM-PE-). The three subject groups were matched for age, diabetes duration, HbA1C, and parity, as appropriate. Diabetic women were entirely complication-free before pregnancy. All study visits took place before the clinical onset of PE.

Results: Plasma concentrations of both ACE and prorenin were similar in the DMPE+ vs. DMPE- women at all three trimesters, but angiotensinogen was significantly lower in DMPE+ than DMPE- subjects at the 3rd trimester (P<0.01). Interestingly, all diabetic women, whether they subsequently developed PE or not, showed significantly higher levels of ACE and lower levels of angiotensinogen at the 1st trimester compared with non-diabetic controls (P<0.01), suggesting increased activity of the renin-angiotensin pathway.

Conclusion: Plasma levels of ACE, angiotensinogen, and prorenin cannot be used for early prediction of PE in women with T1DM. However, the renin-angiotensin system appears to be abnormally active early in pregnancy in women with T1DM, creating 'fertile soil' for the subsequent development of PE.

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1119

Impact of parity on glycaemic control, cardiovascular risk factors and diabetes-related chronic complications: a multicentre study in BrazilC.A. Negrato¹, M.B. Gomes²¹Internal Medicine, Bauru's Diabetics Association, Bauru,²Diabetes Unit, State University of Rio de Janeiro, Brazil.

Background and aims: Pregnancy in women with preexisting diabetes can predispose these women to some diabetes-related chronic complications or accelerate the course of these complications if they are already present. The aim of this study was to determine the impact of parity on glycemic control, cardiovascular risk factors and diabetes-related chronic complications in women with type 1 diabetes in Brazil.

Materials and methods: This was a multicenter cross-sectional study conducted between December 2008 and December 2010 in 28 public clinics in 20 cities from the four Brazilian geographic regions. Data were obtained from 1,532 female patients, 59.2% Caucasian, aged 25.2 ± 10.6 years. The diabetes mean duration was 11.5 ± 8.2 years. Patient information (clinical factors and number of pregnancies) were obtained through a questionnaire and a chart review. Number of pregnancies were stratified in five groups: group 0 (no pregnancy), group 1 (one pregnancy), group 2 (two pregnancies), group 3 (three pregnancies) and group 4 (\geq four pregnancies).

Results: The comparison between the patients stratified according to parity showed that patients from groups 0, 1 and 2 were younger than patients from group 4 ($p < 0.001$). Patients from groups 0 and group 1, had been diagnosed with diabetes with lower age and had less mean diabetes duration than patients from the other groups ($p < 0.001$). The age at menarche was not different between the four groups ($p = 0.3$). Overweight or obesity was present in 538 patients (35.1%). Patients from group 0 had lower BMI than patients from the other groups. A linear association was found between the frequency of overweight or obesity and parity ($p = 0.004$). A difference between the five groups was observed for the mean of HbA1c without difference between-groups. A higher frequency of hypertension and higher levels of systolic and diastolic blood pressure were observed in group 4 in comparison to the other groups ($p < 0.01$ for all comparisons). Metformin was used by 162 patients (10.6 %) and its use was related to parity ($p = 0.02$). The number of pregnancies was related to the presence of diabetes-related chronic complications (micro and macrovascular). A lower frequency of non-proliferative and proliferative retinopathy and macrovascular complications was noted in patients from group 0 in comparison to the other groups ($p < 0.01$). A tendency for an association between parity and nephropathy was also observed ($p = 0.056$).

Conclusion: Parity in women with type 1 diabetes is strongly associated with the presence of cardiovascular risk factors and diabetes-related chronic complications. Further prospective studies must be addressed to establish the relationship between the impact of pregnancy upon starting and/or deteriorating cardiovascular risk factors and diabetes-related chronic complications.

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Association between breast-feeding duration with post-pregnancy glycaemic control and body weight in women with type 1 diabetesM.T. Małecki¹, B. Katra¹, K. Cyganek¹, P. Blizanowska², B. Matejko¹¹Department of Metabolic Diseases,²Department of Pharmacy, Jagiellonian University, Kraków, Poland.

Background and aims: In accordance with the new WHO guidelines, breast-feeding (BF) is recommended until the sixth month of the baby's life. Such model of feeding reduces the risk of digestive tract infections with infants and their susceptibility to illnesses. Apart from obvious benefits for the infant's health, it has also beneficial effect on the mother's health. BF may be more challenging in women with type 1 diabetes (T1DM) because of difficulties in maintaining their glycemic control. The frequency of long-term BF in T1DM women and its relationships with clinical course of T1DM is unknown.

Materials and methods: The aim of the study was assessment of frequency of long BF (defined as 6 months or longer) in T1DM women and its impact on post-pregnancy glycemic control as well as body weight. We collected data on BF from singleton pregnancies in 176 consecutive women with T1DM (mean age 27.8 yrs, mean BMI 23.3, mean T1DM duration 11.9) who were under our medical care between 1999–2012. They were contacted to complete a standard questionnaire on BF. Their medical records were available for the analysis. We divided all post-pregnancy follow-up data into three groups:

within 6 months after delivery (period I), 6–12 months after delivery (period II), and more than 12 months (period III).

Results: The majority of T1DM women ($n = 110$, 62.5%) breastfed for at least 6 months. The clinical characteristics of long-term BF T1DM women vs. short-term BF T1DM women was as follow: mean age - 28.4 yrs. vs. 26.7 ($p = 0.01$), mean T1DM duration - 11.9 yrs. vs. ± 11.4 ($p = 0.64$), mean BMI before pregnancy - 23.5 kg/m² vs. 23.6 ($p = 0.64$), proportion of pregnancy planning - 68% vs. 56 ($p = 0.10$). The HbA1c level in the long-term BF was lower at period II and III of the post-pregnancy follow-up (6.0% vs. 7.7% $p = 0.05$; 6.9% vs. 7.9%; $p = 0.02$, respectively). BMI did not differ between long-term BF and short-term BF group at any follow-up period.

Conclusion: Long-term BF seems to be associated with lower HbA1c, but not BMI, after the pregnancy. Type 1 diabetes and breast-feeding require a change in the habits and routines of everyday life. This results could be a better treatment motivation in this group of patients.

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Presence of components of the metabolic syndrome in offspring of mothers with type 1 diabetesZ. Vlachová¹, B. Bytøft², S. Knorr³, T.D. Clausen⁴, R. Beck-Jensen⁵, K. Højlund¹, E.R. Mathiesen⁶, P. Ovesen⁷, H. Beck-Nielsen¹, C.H. Gravholt³, P. Damm², D.M. Jensen¹

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Background and aims: Some studies have shown that intrauterine exposure to maternal type 1 diabetes (T1DM) may have long-term effects on the offspring including increased risk of obesity and type 2 diabetes. However, the knowledge of the long-term impact of various maternal glycaemic levels during pregnancy is scarce. We aimed to study body composition and other metabolic risk factors in offspring of T1DM mothers compared to offspring of non-diabetic mothers with specific focus on associations between maternal glycaemic control during pregnancy and long-term outcomes of the offspring.

Materials and methods: During 1993 to 1999 pregnancies of women with T1DM in Denmark were prospectively reported to a central registry in the Danish Diabetes Association ($n = 1215$). Maternal glycaemic status along with pregnancy outcome and complications were recorded. We invited 746 children from these pregnancies to a follow-up examination. A group of control children matched with respect to date of birth, sex and postal code was identified from the National Central Person Registry. Anthropometric measurements and blood sampling for metabolic characterization inclusive an oral glucose tolerance test were performed.

Results: We examined 277 children of women with T1DM (mean age 16.7 (13.0 - 19.8 years)) and 304 control children (mean age 16.8 (13.5 - 20.4 years)). Offspring of women with T1DM had significantly higher body mass index standard deviation score (BMI-sds) based on Danish reference curves for BMI adjusted for age and sex (Δ BMI-sds: 0.46 (95% CI; 0.27 - 0.65)), waist circumference (Δ : 3.6; 2.4 - 4.9 cm), waist-to-hip ratio (Δ : 0.009; 0.002 - 0.016), fasting serum insulin (Δ : 6.9; 2.0 - 11.6 pmol/l) and 2 h serum insulin (Δ : 43.5; 14.4 - 72.7 pmol/l) compared to control offspring. Furthermore we observed that offspring of T1DM mothers with peri-conceptual HbA1c levels above 8 % (63 mmol/mol) had higher BMI, waist circumference and 2 h serum insulin than offspring of mothers whose peri-conceptual HbA1c was below 7 % (53 mmol/mol).

Conclusion: Offspring of mothers with T1DM have a more unfavourable metabolic profile than children of non-diabetic mothers. Our data indicate that maternal intrauterine hyperglycemia plays a significant role in this.

Clinical Trial Registration Number: NCT01559181

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PS 097 Neuropathy: biomarkers and mechanisms

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Cardiovascular autonomic tone relation to obesity stage, metabolic syndrome, advanced glycation end products and other metabolic parameters in prediabetes

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Background and aims: The aim of the study was to assess cardiovascular autonomic function (CAF) at different stage of obesity and in the presence of metabolic syndrome (MetS), and its relation to metabolic parameters and advanced glycation end products (AGEs) in subjects with prediabetes - impaired fasting glucose (IFG) and impaired glucose tolerance (IGT).

Materials and methods: A total of 148 subjects - 91 females and 57 males (mean age 50.4 ± 13.6 years, mean BMI $32.4 \pm 8.8 \text{ kg/m}^2$) with prediabetes - 83 with IFG, 29 with IGT and 36 with IFG+IGT, divided into 5 groups according to BMI: 15 normal weight, 43 overweight, 48 obesity class I, 25 class II, and 17 class III, and into 2 groups according to the presence of MetS - 118 with MetS and 30 controls were enrolled. Glucose tolerance was studied during OGTT. Anthropometric indices, blood pressure and serum lipids were measured. AGEs were evaluated by skin autofluorescence (AGE-Reader). Body composition was estimated by impedance analysis (InBody 720). CAF was assessed by ANX-3.0 using frequency-domain analysis during standard clinical tests - deep breathing, Valsalva and standing from a seated position.

Results: No statistically significant difference in CAF was established between the groups according to BMI and the presence of MetS, as well as between subgroups according to glucose intolerance. In subjects with prediabetes sympathetic and parasympathetic tone at rest and during applied clinical tests showed negative correlation with visceral fat area, AGEs, and HbA1c and did not demonstrate significant correlation with BMI, waist circumference, lipid profile and blood pressure.

Conclusion: Obesity stage and MetS are not associated with CAF deterioration in prediabetes on their own. Central obesity, HbA1c, and AGEs accumulation appear to be the main determinants of autonomic imbalance and increase cardiovascular risk at the early stages of glucose intolerance.

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Decreased beta cell function is associated with cardiovascular autonomic neuropathy in newly diagnosed type 2 diabetic patients

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is closely associated with increased mortality in diabetic patients. Several risk factors of CAN have been clearly clarified. However, the impact of beta-cell function on CAN, especially in newly diagnosed type 2 diabetic patients, is still controversial. Therefore, this study aimed to investigate the association between beta-cell function and CAN in newly diagnosed type 2 diabetic patients.

Materials and methods: 90 newly diagnosed type 2 diabetic patients (68.9% male, mean age: 45 years old) were enrolled. Standard battery of Ewing tests, including Valsalva manoeuvre (Valsalva ratio), the deep breathing test of expiration-to-inspiration ratio and the lying to standing test among the heart rate tests, and the orthostatic hypotension test, were used to identify CAN. Based on the results from Ewing tests, subjects were then further divided into two groups: diabetic patients with CAN (CAN+ group) or without CAN (CAN- group). Fasting glucose, insulin and c-peptide were measured. HOMA-B and HOMA-IR were calculated.

Results: The positive rate of CAN in newly diagnosed type 2 diabetic patients was 22.2%. Compared with CAN- group (n=70), CAN+ group (n=20) had significantly lower fasting insulin, c-peptide and HOMA-B [median (interquartile range): $5.34(3.50\sim 8.57) \text{ mu/L}$ VS $8.75(4.92\sim 13.87) \text{ mu/L}$, $P=0.045$; $0.51(0.39\sim 0.65) \text{ nmol/L}$ VS $0.75(0.51\sim 0.98) \text{ nmol/L}$, $P=0.14$;

$15.75(9.52\sim 36.36)$ VS $32.28(18.67\sim 61.04)$, $P=0.012$, respectively]. No significant difference was observed in HOMA-IR between groups. Fasting c-peptide was correlated with Valsalva ratio ($r=0.24$, $P=0.043$) and the lying to standing test ($r=0.26$, $P=0.023$). Logistic regression analysis revealed that HOMA-B was an independent protective factor of CAN in this population (OR: 0.97, 95%CI: 0.94~0.99, $P=0.027$).

Conclusion: Prevalence of cardiovascular autonomic nerve is high in newly diagnosed type 2 diabetic patients. Decreased beta-cell function is associated with CAN in this population.

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1124

Autonomic dysfunction is associated with loss of postprandial glycaemic control in newly diagnosed type 2 diabetes patients

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Background and aims: Maintaining tight blood glucose control by minimizing the magnitude and frequency of glucose excursions and especially postprandial glucose control is essential in achieving an ideal glycaemic control. Glycaemic variability may play a significant role in the development of diabetic neuropathy e.g. through activated oxidative stress. The aim of the present study was to investigate the association between autonomic dysfunction and postprandial glucose control in newly diagnosed type 2 diabetes patients.

Materials and methods: A total of 81 patients diagnosed within the last 5 years with type 2 diabetes according to WHO-criteria were consecutively recruited for this observational cohort study. All patients were equipped with a continuous glucose monitor (CGM) for three days and performed self-monitored blood glucose measurements (SMBG) at least four times per day during this period. Afterwards they were all tested for cardiac autonomic neuropathy (CAN) using conventional cardiovascular reflex tests (response to standing, deep breathing and Valsalva manoeuvre) and divided according to the presence of CAN. Postprandial glucose measures were calculated using CGM for each group based on the time of SMBG before main meals.

Results: The cohort consisted of 38 women and 43 men with non-insulin-treated type 2 diabetes, a mean age of $58 (\pm 11)$ years, a BMI of $30 (\pm 4)$, and an HbA1c of $6.6 (\pm 0.7) \%$. CAN was present in 22 subjects (9 women and 13 men). The groups with and without CAN were similar with respect to blood pressure, HbA1c, cholesterol levels and had comparable smoking habits. The nocturnal glucose drop and dawn glucose levels were significantly higher in the group with CAN as compared with patients without CAN (7.4 versus 6.5 mmol/L, $P=0.017$) and (7.9 versus 7.2 mmol/L, $P=0.045$). The breakfast premeal glucose was not different between patients with or without CAN, but peak glucose after breakfast was significantly higher in patients with CAN (11.4 versus 9.7 mmol/L, $P=0.009$), and the autonomic dysfunction group had significantly larger excursions 0.5-1.5 hour post meal ($P<0.04$, Figure 1). No difference in glucose was seen between groups at lunch or dinner. After adjusting for gender, age, systolic blood pressure, resting heart rate and BMI, the presence of CAN still had a significant influence on breakfast peak glucose ($P=0.005$) and the postprandial breakfast glucose increase.

Conclusion: CAN is associated with loss of postprandial control in newly diagnosed type 2 diabetes patients. These findings emphasise the importance of screening for autonomic changes early in diabetes and the need for under-

standing the complex interaction between postprandial glucose excursions and the development of autonomic neuropathy.

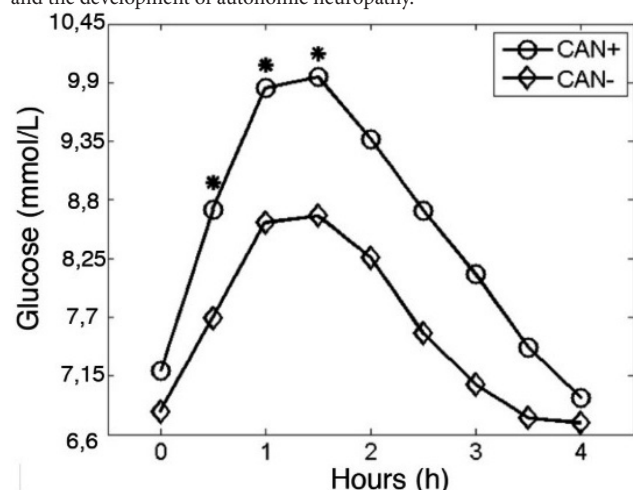


Figure 1 - Postprandial excursions 0-4 hours post breakfast in subjects with and without CAN. Star (*) indicates significant differences between groups

Clinical Trial Registration Number: NCT00674271

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Artery stiffness parameters in patients with cardiovascular autonomic neuropathy and type 2 diabetes mellitus

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Background and aims: Artery stiffness parameters are known as a potentially applicable atherosclerotic risk marker and strongest predictors of cardiovascular morbidity and mortality. This study was aimed to investigate the parameters of arterial stiffness in patients with type 2 diabetes mellitus (DM) and cardiovascular autonomic neuropathy (CAN).

Materials and methods: The study involved 65 patients with type 2 DM of age 54.7 ± 3.8 years, median BMI 28.5 ± 0.9 kg/m² and HbA1c level $7.4 \pm 0.6\%$, among them 12 patients without cardiovascular diseases (CVD) and CAN, 14 patients with mild, 18 - moderate and 21 severe CAN. Control - 12 healthy volunteers. The diagnosis of CAN was based on the results of 5 standard cardiovascular tests, 24-hour HRV. The 24-hour blood pressure profiles, aorta (AIxao) and brachial augmentation index (AIxbr), pulse wave velocity (PWV) and ambulatory arterial stiffness index (AASI) were assessed by TensioMedTM Arteriograph 24 (Hungary). Statistics: ANOVA.

Results: We found that development of mild CAN in patients with type 2 DM is associated with statistically significant increase of artery stiffness indices (AIxao +22.1%, AIxbr +41.9%, AASI +16.7%, $p < 0.05$; PWV +13.5%, $p < 0.01$) compared to patients without verified CVD and CAN. The average value of PWV was considered as high (10.1 m/sec). Among the patients of this group in 7.1% we observed the optimal value of AIxbr, in 50.0% - normal, 42.9% - elevated; in 42.9% - normal value of PWV and in 57.1% - elevated. The arterial stiffness parameters in patients with moderate CAN exceed the physiological values, in particular AIxao +26.2%, $p < 0.01$; AIxbr +66.2%, $p < 0.001$; PWV +24.7%, $p < 0.001$; AASI +30.6%, $p < 0.01$ compared to group without CVD and CAN. PWV was statistically significant higher compared to patients with mild CAN (+9.9%, $p < 0.05$). The value of AIxbr was normal in 55.5%, elevated in 38.9%, pathological in 5.6%; PWV in 16.7% - normal, 50.0% - elevated and in 33.3% of cases pathological. The development of severe CAN was characterized by elevated and pathological values of arterial stiffness parameters, in particular AIxao +37.8%, AIxbr +81.2%, PWV +37.1% and AASI +55.6%, $p < 0.001$ compared to patients without CVD and CAN. The values of PWV and AASI were statistically significant higher compared to patients with mild (+20.8% and +33.3%, $p < 0.001$) and moderate CAN (+9.9% and +19.2%, $p < 0.05$). Among the patients of this group mostly elevated and pathological parameters were observed: in 42.9% - elevated parameters of AIxbr; in 23.8% - PWV; in 38.1% - pathological levels of AIxbr; 61.9% - PWV; normal value of PWV was observed in 14.3% of patients, AIxbr in 19.0% (table).

Conclusion: The development and progression of CAN is associated by increasing of artery stiffness parameters, in particular mild CAN by elevated PWV, moderate - by elevated and severe by elevated and pathological values of all investigated parameters. Non-invasive arteriography may be included in complex algorithm for CAN diagnosis and reduction of artery stiffness parameters should be one of the treatment goals.

Parameters	Control (n=12)	Patients with type 2 DM (n=65)			
		Without and CVD CAN (n=12)	CAN stage		
Groups	A	B	Mild (n=14) C	Moderate (n=18) D	Severe (n=21) E
AIxao, %	20.6±1.71	26.7±1.84 $p < 0.05$	32.6±1.76 $p < 0.001$ $p_1 < 0.05$	33.7±1.24 $p < 0.001$ $p_1 < 0.001$ $p_2 > 0.05$	36.8±1.57 $p < 0.001$ $p_1 < 0.001$ $p_2 > 0.05$ $p_3 > 0.05$
AIxbr, %	-33.7±2.86	-23.4±1.91 $p < 0.01$	-13.6±3.67 $p < 0.001$ $p_1 < 0.05$	-7.9±2.67 $p < 0.001$ $p_1 < 0.001$ $p_2 > 0.05$	-4.4±3.08 $p < 0.001$ $p_1 < 0.001$ $p_2 > 0.05$ $p_3 > 0.05$
PWV, m/s	7.2±0.31	8.9±0.25 $p < 0.001$	10.1±0.24 $p < 0.001$ $p_1 < 0.01$	11.1±0.39 $p < 0.001$ $p_1 < 0.001$ $p_2 < 0.05$	12.2±0.29 $p < 0.001$ $p_1 < 0.001$ $p_2 < 0.001$ $p_3 > 0.05$
AASI	0.3±0.02	0.36±0.02 $p < 0.05$	0.42±0.02 $p < 0.001$ $p_1 < 0.05$	0.47±0.03 $p < 0.001$ $p_1 < 0.01$ $p_2 > 0.05$	0.56±0.03 $p < 0.001$ $p_1 < 0.001$ $p_2 < 0.001$ $p_3 < 0.05$

p – compared to group A; p₁ – group B; p₂ – group C; p₃ – group D. Data are mean ± SEM.

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Low serum ficolin-3 levels indicate peripheral neuropathy in diabetic patients

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Background and aims: Diabetic peripheral neuropathy (DPN) is one of the most common chronic complications of diabetes. The inflammatory mechanisms involving complement activation has been shown to take part in the pathophysiology of DPN. Ficolin-3, a soluble molecule of the innate system, has a primary role in the activation of the lectin pathway in the complement system. In the present study we aimed to elucidate the possible connection between serum ficolin-3 levels and DPN in diabetes mellitus.

Materials and methods: Consecutive diabetic inpatients at the Shanghai Clinical Medical Center of Diabetes from January 2012 to December 2013 were randomly recruited in this cross-sectional study. The demographic and clinical variables were obtained through a questionnaire. Serum ficolin-3 concentration was measured with ELISA. DPN was diagnosed by abnormalities in two or more of three major categories (positive symptoms, increased vibration perception threshold (VPT) values, and slowed nerve conducting velocities (NCVs)). Patients were divided into two groups including DPN and non-DPN group.

Results: A total of 466 patients (mean age, 57.98 ± 11.09 years; male/female, 256/210; mean diabetes duration, 9(4-15) years) were finally enrolled in this study. There was no difference in serum ficolin-3 levels in male and female patients (25.00 ± 11.52 vs 23.74 ± 12.27 ng/mL, $P = 0.257$), also no difference was found in the prevalence of DPN in male and female patients. There were significantly difference in ficolin-3 levels among patients without diabetic complications ($n = 156$, 27.15 ± 10.23 ng/mL), complicated with only DPN ($n = 40$, 18.30 ± 8.99 ng/mL), only diabetic nephropathy ($n = 25$, 24.78 ± 9.63 ng/mL), as well as only diabetic retinopathy group ($n = 58$, 25.27 ± 10.26 ng/mL) ($P < 0.001$). Serum ficolin-3 levels were lower in DPN patients compared with non-DPN patients (18.73 ± 10.75 vs 26.69 ± 10.68 ng/mL, $P < 0.001$). Ficolin-3 levels were negatively correlated to VPT ($r = -0.158$, $P = 0.025$). Partial spearman correlation analysis further indicated that ficolin-3 was still associated with VPT ($r = -0.283$, $p = 0.001$) even after adjusted for age, diabetes duration, smoking, hypertension, alanine aminotransferase and Glomerular filtration rate. Logistic regression analysis of DPN showed that age (OR, 1.047; 95%CI 1.005-1.092; $p = 0.029$), diabetes duration (OR, 1.10; 95%CI 1.040-1.166; $p = 0.001$), ficolin-3 (OR, 0.939; 95%CI 0.910-0.970; $p < 0.001$) were independent associated factors for DPN. The prevalence of DPN among ficolin-3 quartiles was compared too, and the results revealed that it's elevated with the descending serum ficolin-3 concentrations. Par-

ticipants in the lowest quartile of serum ficolin-3 had a significantly increase risk of DPN compared with those belongs to the highest quartiles. Compared with Quartile1(referent), patients in Quartile2(OR, 2.76; 95%CI, 1.56-4.88; $P<0.001$), Quartile 3(OR, 3.02; 95%CI, 1.69-5.40; $P<0.001$) and Quartile4(OR, 6.84; 95%CI, 3.39-13.80; $P<0.001$) had higher risk of DPN.

Conclusion: Ficolin-3 levels decrease in diabetic patients with DPN, strongly inversely correlated with VPT. The risk of DPN increased with the decrease of serum ficolin-3 levels. Therefore, decreased ficolin-3 level may be a biomarker of DPN in diabetic population.

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1127

High serum cystatin C indicates diabetic peripheral neuropathy in type 2 diabetes

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Background and aims: Recent studies have shown that cystatin C is a predictor of mortality and cardiovascular diseases. We carried out this study to explore the relationship between serum cystatin C and diabetic peripheral neuropathy(DPN)

Materials and methods: Totally 937 diabetic patients were randomly enrolled in this cross-sectional study. The demographic and clinical variables were obtained through a questionnaire. Serum cystatin C concentration was measured with immunoturbidimetry. DPN was evaluated by neurological symptoms, neuro-thesiometer and electromyography.

Results: Serum cystatin C levels were higher in confirmed DPN patients[1.3(1.1-1.5)mg/L] compared with unconfirmed DPN[1.1(0.9-1.3) mg/L, $p<0.001$] and non-DPN patients(1.0(0.9-1.3)mg/L, $p<0.001$). Spearman correlation analysis showed that confirmed DPN was positively related to age($r=0.196$, $p<0.001$), diabetes duration($r=0.141$, $p<0.001$), hypertension($r=0.128$, $p<0.001$), CysC($r=0.154$, $p<0.001$), Cr($r=0.066$, $p<0.05$) and urinary albumin ($r=0.144$, $p<0.001$), and negatively related to GFR($r=-0.164$, $p<0.001$). Patients were then divided into quartiles according to the serum CysC levels. Compared with cystatin C Quartile1(referent), the risk of confirmed DPN was significantly higher in Quartile2(OR, 1.753; 95%CI 1.055-2.912; $p<0.05$), Quartile3(OR, 2.463; 95%CI 1.445-4.917; $p<0.001$) and Quartile4(OR, 5.867; 95%CI 2.075-16.589; $p<0.001$). Finally, receiver operating characteristic (ROC) analysis revealed that the optimal cutoff point of cystatin C to predict DPN(OR, 2.380; 95%CI 1.577-3.592; $p<0.0001$) in diabetic patients without diabetic nephropathy was 1.25mg/L(AUC=0.704, 95 % CI 0.645-0.763, Youden index=0.305, sensitivity: 61.8%, specificity: 68.6%).

Conclusion: Cystatin C levels are higher in diabetic patients with DPN, and high serum cystatin C may be a risk factor and potential biomarker for diabetic peripheral neuropathy.

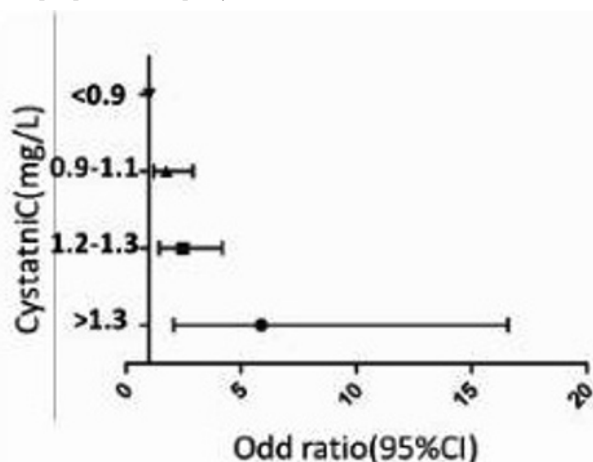


Figure1 DPN risk in different CysC quartiles.

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1128

GABA-dependent pain facilitation of spinal 5-HT3 receptor in diabetic neuropathic pain

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Background and aims: Diabetic neuropathic pain (DNP) is a frequent complication of diabetes, associated with increased morbidity and reduced quality of life. Impaired spinal pain inhibition is likely to contribute to DNP, namely the increased GABA content and the shift in its role from inhibitory to excitatory through its action on GABA A receptors (GABAAR). The activation of spinal 5-HT3 receptor (5-HT3R) is known to induce GABA release and its inhibition was shown to be anti-hyperalgesic in streptozotocin (STZ)-diabetic rats. We hypothesised that the anti-hyperalgesic effect of 5-HT3R inhibition is mediated by counteracting the excitatory role of GABA through GABAAR at the spinal cord of STZ-diabetic rats. In this study we evaluated the effect of intrathecal administration of muscimol (GABAAR agonist) on the mechanical hyperalgesia reversal induced by 5-HT3R inhibition.

Materials and methods: Diabetes was induced in male Wistar rats by intraperitoneal injection of STZ solution (60 mg/Kg). Control animals received only the vehicle. Blood glucose concentration was quantified in blood samples taken from the tail vein and only STZ-injected rats with glycaemia higher than 270 mg/dl were considered diabetic and included in the study. At 4 weeks post-injection, a catheter was implanted in the lumbar spinal cord subarachnoid space for drug administration. Mechanical nociception was evaluated by paw-pressure test in control and STZ-diabetic rats after intrathecal delivery of saline (STZ+saline; ctr+saline) or Y-25130 hydrochloride (Y-25130, 5-HT3R antagonist, 30 fmol, STZ+Y-25130; ctr+Y-25130). An additional group of STZ+saline and STZ+Y-25130 diabetic rats received intrathecal administrations of muscimol (0.3 ug) at 3,5h after saline or Y-25130 infusions(STZ+saline+musc; STZ+Y-25130+musc) and the PPT were evaluated 30 min after. Means were compared by ANOVA repeated measures or one-way ANOVA followed by Tukey post-hoc test and are presented as mean±SEM.

Results: The STZ-diabetic rats presented significantly increased blood glucose levels and decreased body weights at 4 weeks after diabetes induction when compared with control rats. The STZ-diabetic rats developed mechanical hyperalgesia as demonstrated by the significantly lower paw pressure threshold (PPT) when compared with control animals (STZ: 5.74 ± 0.71 ; control: 10.42 ± 0.20 , $p<0.001$). Intrathecal delivery of Y-25130 in STZ-diabetic rats significantly increased the PPT, with a maximum peak effect at 4h post-administration (STZ+saline: 6.10 ± 0.32 ; STZ+ Y-25130: 10.98 ± 0.74 , $p<0.01$). The administration of muscimol 30 min before the peak maximum effect of Y-25130 abolished its anti-hyperalgesic effect in STZ-diabetic rats (PPT in STZ+Y-25130: 10.98 ± 0.74 ; STZ+Y-25130+Musc: 5.70 ± 0.64 , $p<0.01$).

Conclusion: These findings suggest that the activation of 5-HT3R at the spinal cord of STZ-diabetic rats is likely to increase GABAergic neurotransmission. Considering the previously reported shift in the role of GABA, through the activation of GABAAR, in STZ-diabetic rats, this is likely to be facilitating pain spinal transmission. The use of 5-HT3R antagonists may then be considered as a promising pharmacological treatment in alleviating mechanical hyperalgesia induced by diabetes.

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1129

Glucosamine induced Schwann cell death and nerve conduction delay associated with activation of hexokinase

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Background and aims: Glucosamine is one of the amino-sugars, widely sold on the market for relief of symptoms in patients with arthritis. In our previous studies, comprehensive metabolomics analysis enabled us to find accumulation of glucosamine (GlcN) in the sciatic nerve of STZ-diabetic mice. GlcN is known to metabolize to GlcN-6-phosphate via hexokinase (Hex), but its metabolic sequelae is not well understood. In this study, we therefore ex-

amined whether excessive GlcN-Hex flux influences on Schwann cell survival in vitro and peripheral nerve function in vivo.

Materials and methods: Schwann cell line (IMS32) maintained in DMEM supplemented with 10% FBS in 5% CO₂ was stimulated with 10 mM GlcN. Western blot was conducted for the evaluation of apoptosis to identify cleaved caspase3 (CC3) and for the expression of modified glycosylation (O-GlcNAc). Hex activation was separately examined in the fractionated mitochondria and cytosole of IMS32 exposed to GlcN. Specific siRNA for Hex I was applied to see the effects of the Hex I on GlcN-Hex pathway on Schwann cell survival. To see the effects of GlcN on Hex expression in the peripheral nerve in vivo, male C57Bl/6J mice, 12 weeks of age were administered (i.p.) with 2 g/kg GlcN and nerve conduction velocity was measured. A half of the animals were pretreated with inosine (100 mg/kg, i.p.), as an ATP donor, one hour prior to GlcN administration. Normal control mice were given saline alone to serve for comparison.

Results: In vitro experiment revealed that GlcN exposure elicited increased CC3 expression in Schwann cells. Concurrently, O-GlcNAc expression was increased with GlcN exposure, but it was not proportional to the extent of apoptosis. Hex I expression in mitochondria fraction was markedly increased after GlcN exposure irrespective of glucose concentration. Knock down of Hex I by siRNA suppressed the expression of GlcN-induced CC3 activation and caused prolonged cell survival. Mice injected with GlcN exhibited enhanced Hex expression of mitochondrial fraction and delayed nerve conduction, which was prevented by pretreatment with inosine.

Conclusion: GlcN exposure induced Schwann cell death with Hex activation in vitro and nerve conduction delay in vivo. Inhibition of Hex activation by siRNA in vitro and inosine pretreatment significantly inhibited GlcN-induced Schwann cell death or neuropathic changes. These findings suggest that GlcN-Hex activation may have a pathogenetic role in diabetic neuropathy.

PS 098 Mechanisms and outcomes of neuropathy

1130

Glycaemic variability as measured by continuous glucose monitoring and diabetic cardiovascular autonomic neuropathy in patients with type 2 diabetes

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Background and aims: It is still not clear whether glycemic variability contributes to the development of diabetic autonomic neuropathy. We investigated the association between glycemic variability determined by continuous glucose monitoring (CGM) and diabetic cardiovascular autonomic neuropathy in the patients with type 2 diabetes.

Materials and methods: A total of 184 patients with type 2 diabetes with estimated glomerular filtration rate of >30ml/min/1.73m² who wore a CGM device for 72 hours were enrolled. As parameters of glycemic variability, the standard deviation (SD) around the mean and the coefficient of variation (CV, SD/mean) were obtained from the CGM data. All subjects were investigated for five standard cardiovascular reflex tests according to the Ewing's protocol. Diabetic cardiovascular autonomic neuropathy was defined as the presence of at least two abnormal tests or autonomic neuropathy points ≥2.

Results: Among the enrolled patients, 97 had diabetic cardiovascular autonomic neuropathy (52.7%, neuropathy group). SD (49.76±19.28 vs 46.05±21.24, P=0.22) and CV (28.63±8.24 vs 28.33±9.88, P=0.81) did not differ between neuropathy and control group. The hemoglobin A1c (HbA1c, %) was higher in neuropathy group (8.43±1.48 vs 7.95±1.32, P=0.02) than in the control group. The duration of diabetes was longer in neuropathy group than in control group (12.49±7.52 vs 6.72±6.72 year, P=0.048). In binary logistic regression analysis, CV and SD failed to contribute to the final model.

Conclusion: We failed to show an independent association between glycemic variability as measured by CGM and diabetic cardiovascular autonomic neuropathy in the patients with type 2 diabetes.

1131

Effects of diabetic peripheral neuropathy on the use of vision during walking

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Background and aims: Due to a decrease in sensory feedback, patients with diabetic peripheral neuropathy (DPN) are expected to use vision as a way of detecting foot position during walking, restricting their ability to visually identify upcoming obstacles. By examining how people visually acquire (first look at) targets during walking, we can elucidate to what extent diabetes patients use their eyes to identify foot position, and plan subsequent stepping during walking.

Materials and methods: Twelve participants (4 with diabetic peripheral neuropathy [DPN], 4 with non-neuropathic diabetes [D], and 4 healthy controls [C]) negotiated a stepping walkway, stepping on irregularly placed targets as accurately as possible whilst walking at a natural gait velocity. The timing of horizontal eye movements during stepping were measured using an eye-tracking device, with respect to foot-target contact. Mean group differences were analysed using a one-way ANOVA (p<0.05).

Results: Patients with DPN visually acquired the targets significantly later (C: -0.63 ± 0.20; D: -0.41 ± 0.12; and PN: -0.41 ± 0.14s [p<0.05]) (Fig 1.), and remained looking at them for significantly longer after the foot had contacted the ground (C: 0.00 ± 0.08; D: 0.01 ± 0.08; and PN: 0.06 ± 0.14s [p<0.05]). Patients with DPN also spent significantly less time in total looking at targets than the control group (C: 0.62 ± 0.16; D: 0.42 ± 0.15; and DPN: 0.5 ± 0.21s [p<0.05]), but took significantly longer to look between targets (C: 0.1 ± 0.04; D: 0.11 ± 0.06; and DPN: 0.14 ± 0.11s [p<0.05]).

Conclusion: Patients with neuropathy appear to use vision as a method of detecting when foot placement occurs during stepping. Unlike healthy controls, who look to the next target prior to foot-ground contact, patients with neuropathy remain looking at the target until after foot-ground contact, in

an attempt to increase stepping accuracy. Patients with neuropathy also take longer to look from one target to another, which may indicate the presence of motor neuropathy in the extra-ocular muscles. The increased time to look between targets may therefore be an explanation for the later acquisition of the subsequent target. This visual gaze strategy may restrict the ability of patients with neuropathy to identify upcoming obstacles, increasing the chances of tripping and falling.

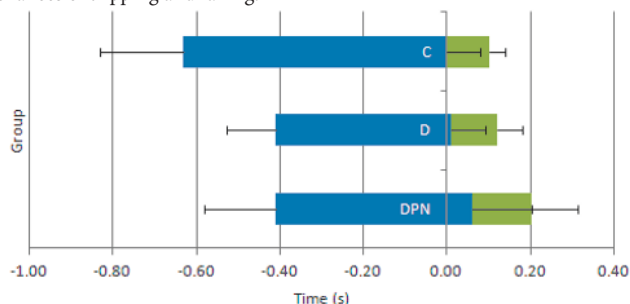


Figure 1. Mean group differences in timing, of when the target was visually acquired (blue bar), and when the eyes were looking from one target to another (green bar), with reference to foot-target contact occurring at 0s.

Supported by: EFSD

1132

Influence of cardiovascular autonomic neuropathy on the next fate of type 1 diabetic patients; 10 years follow up

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is considered to impair morbidity and mortality of diabetic patients (DM). We did not believe in pessimistic results of some studies. Aim of our observational retrospective study was to answer to these questions: 1. In what parameters differed DM with CAN (CAN+) and without CAN (CAN-) at baseline of observation? 2. Did the presence of CAN impair morbidity in next 10 years? 3. Was there the higher mortality among CAN+? What were the reasons of mortality?

Materials and methods: During the years 2002–2004 we evaluated CAN in 278 type 1DM (Ewing battery). CAN+ involved 111, CAN- involved 167 patients. In spite of positive test, majority of patients were asymptomatic. We have compared the control of DM, weight, presence of microangiopathic complications (retinopathy, nephropathy, peripheral neuropathy, diabetic foot) and cardiovascular disease and other risk factors (hypertension, hyperlipoproteinemia and smoking) between groups (CAN+ vs CAN-) at baseline and 10 years after. Median (interquartile range), Wilcoxon non paired test, contingency table, mono- and multifactorial regression and multivariate Regression step-wise were used for statistical evaluation.

Results: 1. Group CAN+ was older (47 vs 33 years; $p<0.001$), with longer duration of DM (20 vs 12 years; $p<0.01$), with worse metabolic control (HbA1c) (73 vs 68 mmol/mol; $p<0.05$), higher systolic (130 vs 120 mmHg; $p<0.001$) and diastolic (80 vs 70 mmHg; $p<0.01$) blood pressure and lower glomerular filtration rate (eGFR) (1.16 vs 1.35 ml/s; $p<0.001$). Retinopathy (67.6 vs 21.6 %; $p<0.001$), neuropathy (59.5 vs 18.6%; $p<0.001$), diabetic foot (6.3 vs 0%; $p<0.01$) and albuminuria (41.4 vs 11.4 %; $p<0.001$) were more frequent in CAN+ at baseline. Same trend was observed in cardiovascular disease (6.3 vs 0.6%; $p<0.01$), hypertension (32.4 vs 6.6%; $p<0.001$) and hyperlipoproteinemia (29.7 vs 16.8 %; $p<0.05$). The ratio of smokers (19 vs 21.6 %) was similar in both groups. 2. During the follow up period there were more new cases of diabetic foot (8.2 vs 0.6%; $p<0.01$) and cardiovascular disease (24.7 vs 3.7%; $p<0.001$) in CAN+. The incidence of other complications and risk factors were similar in both groups: retinopathy: 34 vs 35.9%; albuminuria 17.5 vs 9.2%; neuropathy 29.9 vs 21.5%; hypertension 35.1 vs 30.7%; hyperlipoproteinemia 38.1 vs 29.5%; smoking 1.03 vs 0%. Group CAN+ gained less weight (3 vs 5 kg; $p<0.001$) and the differences in systolic (0 vs 5 mmHg; $p<0.05$) and diastolic (0 vs 0 mmHg; $p<0.05$) blood pressure were smaller, therefore blood pressure in both group were same by the end of the study. The differences in HbA1c did not differ in both groups. 3. In follow up period deceased 18 patients in both groups (18/278; 6.5%), 14 of them from CAN+ (14/111; 12.6%) and 4 from CAN- (4/167; 2.4%) ($p<0.001$). The strongest parameter for mortality was the presence of CAN ($p<0.01$) and systolic blood pressure at baseline ($p<0.05$). Influence of age and duration of DM were insignificant (multivariate

regression step-wise model). The complications of DM (8x), cardiovascular disease (3x) and the other reasons (7x) were the causes of death.

Conclusion: In 1DM patients with CAN the incidence of cardiovascular disease was 7 times, of diabetic foot was 14 times and mortality rate was 5 times higher compared to 1DM without CAN. The incidence of the other complications was similar in both groups.

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1133

Heart rate response to the exercise is impaired even in a mild degree of dysglycaemia

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Background and aims: Impaired heart rate (HR) response to the exercise, chronotropic incompetence, is known to be one of the predictors of prognosis, and it is induced by myocardial ischemia and/or autonomic nervous dysfunction. Although diabetes mellitus is well known to induce the autonomic nervous dysfunction, relationship between chronotropic incompetence and the degree of dysglycaemia is not fully studied yet. We planned to investigate the relationship between them.

Materials and methods: Consecutive 68 angina pectoris patients with complete revascularisation were enrolled. They performed cardiopulmonary exercise test (CPX) and 75g oral glucose tolerance test (OGTT) at the almost the same date. They were divided into two groups according to the result of OGTT (normal glucose tolerance (NGT) group (n=30) and impaired glucose tolerance (IGT) group (n=38)). Chronotropic incompetence was assessed as follows: HR difference between peak and rest (δ HR), HR increase divided by work rate increase (δ HR/ δ WR) and HR recovery 1 minute after exertion (HRR).

Results: All three parameters were smaller in IGT group than NGT group, δ HR: 46.2 ± 3.2 vs. 57.0 ± 3.6 ($p=0.029$), δ HR/ δ WR: 0.53 ± 0.032 vs. 0.66 ± 0.037 ($p=0.011$), and HRR: 16.5 ± 1.5 vs. 21.7 ± 1.6 ($p=0.023$), respectively.

Conclusion: This study clarified that chronotropic incompetence occurs even in the patients with mild dysglycaemia.

1134

Brain perfusion in painful diabetic neuropathy

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Background and aims: Diabetic neuropathy (DN) has been linked to abnormalities in structure and function of the brain on Magnetic Resonance (MR). This study sought to explore intracranial vascular perfusion in patients with type-1 diabetes, with and without painful DN (PDN) and those without diabetes (HV).

Materials and methods: Groups: PDN=7; T1DM without neuropathy (D-NN)=8 & HV=7. MR images were obtained at 3T (Ingenia, Philips Healthcare) using a Dynamic Susceptibility Contrast, T2*-weighted technique (TR/TE=1250/35; 72 dynamics) to assess the passage of a bolus of intravenous gadolinium-chelate passing through the cerebral vascular bed. Contrast perfusion was determined (Nordic NeuroLab ICE, Bergen, Norway) to yield the rBF (cerebral blood flow), rBV (volume) and mTT (mean transit time) in the SC (sensory cortex), Thalami (Thal) and parieto-occipital white matter (POWM). **Results:** Statistically significant differences were observed (ANOVA) between PDN and D-NN group mean mTT as well as PDN and HV group mean mTT [eg. SC-mTT: PDN mean=2.87,SD=0.35; D-NN mean=3.46,SD=0.51; HV mean=3.72,SD 0.73, $p=0.004$; Thal-mTT:PDN mean=2.89, SD=0.48; D-NN mean=3.41,SD=0.55; HV mean=3.82, SD=0.63, $p=0.004$]. BV and BF did not show significant mean differences between the 3 groups.

Conclusion: These early results (from a large on-going study) suggest cerebrovascular perfusion is altered within the brain parenchyma of subjects with PDN when compared to both diabetic and non-diabetic control groups. Microvascular dysfunction may have important implications in our understanding of the brain's involvement in DN, the search for functional neuropathic markers and indicators of future therapeutic intervention. Further work is warranted to further characterise this cerebrovascular involvement.

Supported by: EFSD/Novo Nordisk

1135

Insulin resistance associated interhemispheric coordination deficits in type 2 diabetes mellitus patients: a resting-state study

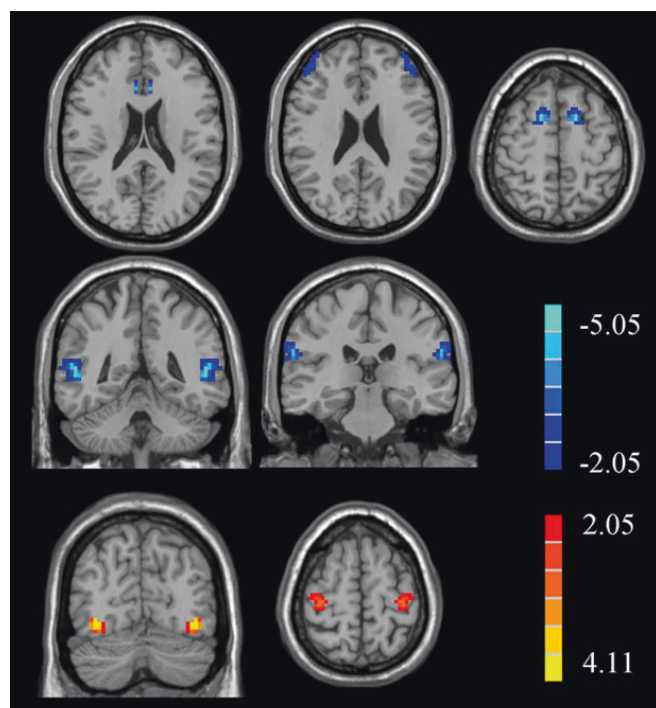
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Background and aims: Type 2 diabetes mellitus (T2DM) has an inextricable link with mild cognitive impairment (MCI) and Alzheimer's disease (AD). T2DM shares several same pathogenesis with AD as well as MCI, which might be mediated by insulin resistance. This study aims to investigate whether decreased interhemispheric coordination exists in T2DM patients using resting-state functional magnetic resonance imaging (rs-fMRI). If so, we further examine whether interhemispheric coordination deficits are associated with the insulin resistance among these patients.

Materials and methods: We compared the interhemispheric resting state functional connectivity of 32 T2DM patients and 30 age-, sex-, and education-matched healthy controls using rs-fMRI, computed using a recently proposed measurement named "voxel-mirrored homotopic connectivity (VMHC)". Pearson's correlation coefficients were measured to detect the relationship between rs-fMRI information and clinical data.

Results: Compared with the healthy controls, the patients showed significant decreases in VMHC in several brain regions within default mode network (DMN). Additionally, the VMHC values in the middle temporal gyrus (MTG) and superior frontal gyrus were found to be correlated inversely with the score of the Trail Making Test-B ($r = -0.404$, $p = 0.027$; $r = -0.544$, $p = 0.002$, respectively) among the T2DM patients. Most importantly, log homeostasis model assessment-insulin resistance (HOMA-IR) had negative correlations with the VMHC values in the MTG among patients ($r = -0.528$, $p = 0.003$).

Conclusion: Patients with T2DM suffer disturbed interhemispheric correlation in several DMN regions, especially in the MTG, which is associated with insulin resistance. We suppose insulin resistance might play a not negligible role in the cognitive dysfunction in T2DM through damaging the MTG function.



Clinical Trial Registration Number: ChiCTR-ONRC-13003095

Supported by: NSFC

1136

Increased grey matter volume loss in diabetic neuropathic subjects with depression

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Background and aims: Using functional magnetic resonance imaging (fMRI), we have demonstrated increased neuronal activation within regions of the brain involved in emotional pain processing. We have also recently reported a significant volume loss in somatosensory cortex peripheral grey matter in patients with both painful and painless diabetic peripheral neuropathy (DPN). As several studies have strongly linked chronic mood disorders (depression and/or anxiety) that are very common in patients with DPN, with brain atrophy, we investigated the relationship between brain volumes and mood disorders in subjects with DPN.

Materials and methods: 24 subjects with type 1 diabetes [No-DPN (n=8), Painful DPN (8) and Painless DPN (8)] underwent detailed neurophysiological assessments to quantify severity of DPN [NIS(LL)+7tests]. All subjects underwent volumetric (0.8x0.8x0.8mm3 resolution) brain MRI at 3T. Images were analysed using FSL (fMRIB, Oxford). Symptoms of depression were assessed using the Hospital Anxiety and Depression Scale (HADS-D).

Results: There were no significant differences in age ($p=0.69$) and duration of diabetes ($p=0.57$) between groups. As expected subjects with painful [9.6(6.6)] and painless [10.2(7.3)] DPN had significantly greater Neuropathy Composite Scores compared the No-DPN group [1.85(1.3); $p=0.02$]. Moreover, subjects with painful DPN had significantly greater depression (HADS-D; $p<0.001$) scores compared to the other study groups. Adjusted peripheral gray matter volume was statistically significantly lower in subjects with painless and painful DPN (mean 599.6 mL [SEM 9.8 mL] and 585.4 mL [10.0 mL], respectively) compared with those with No DPN (626.5 mL [5.7 mL]) and HVs (639.9 mL [7.2 mL]; ANCOVA, $P = 0.001$). We also found a significant negative correlation between both, peripheral grey matter ($p=-0.43$; $p=0.04$) and deep grey matter ($p=-0.51$; $p=0.02$) volumes with HADS-D scores after adjusting for known variables (age, microvascular disease status). There was no significant correlation between HADS-D score and other brain volumes (white matter and CSF).

Conclusion: We have for the first time demonstrated increased grey matter atrophy in DPN subjects with depressive symptoms. Although, previous studies have reported increased brain atrophy in subjects with depression in non diabetic people, this is the first study to demonstrate this association in DPN. Further studies are now required to examine if the brain atrophy related to depression is localised to regions involved in emotional pain processing and the temporal relationship of mood disorders to the neuropathic process.

Supported by: JDRF

1137

Gender differences in impact of self-reported diabetes and neuropathy on quality of life in a large study on Romanian patients

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Background and aims: Objective was to evaluate the gender differences between total and 5 subdomains of Norfolk Quality Of Life Diabetic Neuropathy (QOL-DN) scores in self-reported diabetes with and without neuropathy (DN).

Materials and methods: In this cross-sectional survey, conducted between June and December 2012, 181 healthcare providers from 51 Romanian cities distributed the linguistically translated Romanian Norfolk QOL-DN questionnaire to their patients. Patients completed 35 questions related to their own health perception over the previous 4 weeks, resulting in 21,861 validated forms of 23,543 completed. Total QOL and each domain arithmetic means and standard errors (SEM) were computed.

Results: This is the largest study on gender differences in perception of QOL in patients with self-reported diabetes with and without neuropathy. Female patients reported higher scores than males on Total QOL ($p<0.001$) with greatest impact on AN, symptoms and ADLs in both patients with and with-

out neuropathy. Most important, significantly more male patients (25.1%) than female (20.8%) reported DN complications - foot ulcers, gangrene and amputations ($p<0.001$), although all scores except SFN were significantly higher in women than in men. Both male and females had Total QOL and all 5 subdomains scores significant greater with DN and even greater with ulcers, gangrene or amputations ($p<0.001$).

Conclusion: Diabetes has a greater impact on QOL and all its domains in women than in men based on scores obtained with Norfolk QOL-DN questionnaire. Neuropathy and its complications of foot ulcers, gangrene and amputations greatly worsened QOL in both sexes and reduced but retained differences between the sexes.

Gender differences between Norfolk QOL-DN (Total and Subscale Scores (Mean \pm SEM)) in 21,756 Romanian Patients with Self-Reported Diabetes Mellitus with and without DN Complications

	Total QOL	PFLF	Symptoms	ADL	AN	SFN
ROF	-4-136	-4-56	0-32	0-20	0-12	0-16
ND	(3.8 \pm 0.5)	(0.3 \pm 0.1)	(0.7 \pm 0.1)	(3.1 \pm 0.5)	0.5 \pm (0.1)	(0.6 \pm 0.1)
Diabetes without neuropathy						
Male (n=3396)	11.57 \pm 0.30	6.7 \pm 0.18	2.45 \pm 0.06	0.95 \pm 0.04	0.78 \pm 0.03	0.74 \pm 0.04
Female (n=3219)	15.97 \pm 0.34	9.24 \pm 0.20	3.24 \pm 0.07	1.45 \pm 0.05	1.2 \pm 0.03	0.93 \pm 0.04
p**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Diabetes with neuropathy						
Male (n=6207)	36.51 \pm 0.33	19.25 \pm 0.18	8.32 \pm 0.07	3.83 \pm 0.06	2.08 \pm 0.03	3.24 \pm 0.05
Female (n=7647)	39.92 \pm 0.30	21.23 \pm 0.16	8.96 \pm 0.06	4.29 \pm 0.06	2.43 \pm 0.03	3.25 \pm 0.04
p**	<0.001	<0.001	<0.001	<0.001	<0.001	0.36
Diabetes, neuropathy without foot ulcer(s) gangrene or amputations of toes or fingers						
Male (n=4646)	32.33 \pm 0.36	17.28 \pm 0.20	7.5 \pm 0.08	3.24 \pm 0.06	1.79 \pm 0.03	2.68 \pm 0.05
Female (n=6058)	36.83 \pm 0.32	19.95 \pm 0.17	8.29 \pm 0.07	3.78 \pm 0.06	2.17 \pm 0.03	2.82 \pm 0.04
p**	<0.001	<0.001	<0.001	<0.001	<0.001	0.003
Diabetes, neuropathy with foot ulcer(s) gangrene or amputations of toes or fingers						
Male (n=1561)	48.97 \pm 0.69	25.1 \pm 0.36	10.79 \pm 0.15	5.61 \pm 0.13	2.92 \pm 0.07	4.9 \pm 0.11
Female (n=1589)	51.77 \pm 0.69	26.15 \pm 0.36	11.51 \pm 0.15	6.27 \pm 0.14	3.43 \pm 0.08	4.91 \pm 0.11
p**	0.006	0.04	0.001	0.001	<0.001	0.87

* Quality of life (QOL); Physical Functioning/ large-fiber neurop (PFLF); Activities of daily living (ADL); Autonomic neuropathy (AN); Small-fiber neuropathy (SFN); Range of Scores (ROF); Normative data (ND)

** male vs. female

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PS 099 Diabetic foot: prevention and treatment

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A comparative study of E-Med scan and foot mate print technologies in diabetic neuropathy ascertainment, Dar es Salaam, Tanzania

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Background and aims: In Africa, diabetic foot ulceration is often associated with peripheral neuropathy (PN) and substantial morbidity and mortality. Thus, new affordable technologies for PN screening have the potential to improve patient outcomes across the African continent and other economically less-developed countries. We carried out this study to (i) evaluate two technologies the foot E-med scan system (EMS) and the simple Foot Mate print (FMP) in plantar pressure measurement; and (ii) correlate these measurements with PN ascertained clinically.

Materials and methods: During Jan 2011 - Nov 2013 (study period), all patients attending a diabetes centre in Dar es Salaam, Tanzania, were evaluated clinically following informed consent. Pressure at various sites on each foot was measured with EMS technology and FMP with an ink pad (dark areas reflected high pressures.) PN was ascertained at standard anatomic sites with a thermal sensation machine for pain (warm, heat, cold), monofilament for protective sensation, and biothesiometer for vibration.

Results: Of 1407 patients enrolled during the study period, 801 (60%) were male and 747 (53%) had PN by clinical assessment. Median age =52 (range: 11-90) years; median body mass index=29 (range: 14-59) kg/sq m). Patients with PN were significantly more likely than those without PN to be older (54 vs. 50 years, $p<0.01$) or to have a longer diabetes duration (6 vs. 4 years, $p<0.001$). In addition, per EMS technology, patients with PN were more likely to have significantly higher plantar pressures at the big toe ($p<0.0001$), 3rd, 4th, and 5th toes ($p<0.0001$), central hind foot ($p<0.05$), and mid hind foot ($p=0.005$) versus patients without PN. In contrast, FMP recorded high plantar pressures for PN patients only at the 2nd ($p<0.05$) and 3rd ($p=0.008$) toes.

Conclusion: Pressure measurements were largely predictive of PN. While EMS was more sensitive than FMP in detecting high plantar pressures in patients with underlying PN confirmed by thermal sensation, biothesiometry, and monofilament testing across various anatomic areas of the foot, FMP was sensitive in predicting problems mainly in the toes rather than the hind foot. Despite these differences, both technologies have key major roles to play in the management of diabetic foot complications in Africa and other countries with limited resources.

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The Diabetes Modernisation Initiative (DMI) for Foot Health:

“What does good look like?” Challenges in integrating foot care services across Southwark and Lambeth in London, UK

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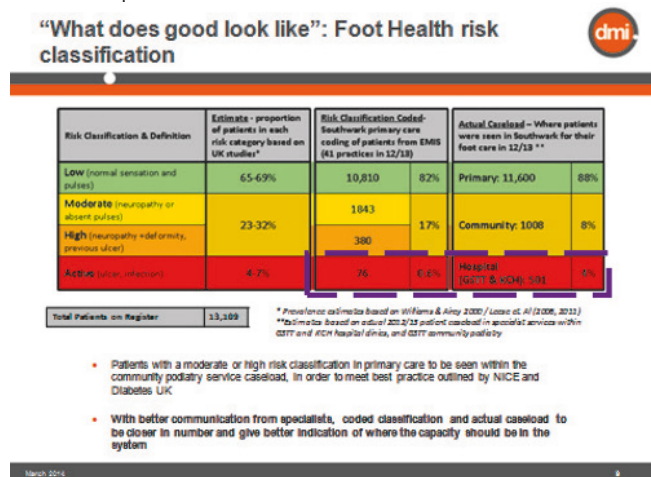
Background and aims: The Diabetes Modernisation Initiative (DMI) in Southwark and Lambeth, two Boroughs in London, set out to bring together a working group of providers and commissioners to explore and develop further foot health improvements across care boundaries. The main areas of focus were to improve the number, quality and accuracy of foot assessments being completed in Primary Care practices. There also was a lack of information as to whether patients were being seen in the most appropriate care setting according to their risk classification.

Materials and methods: The working group in partnership with the Southwark Clinical Commissioning Group (CCG) investigated the risk stratification in primary care against actual patient activity in hospital and community settings in order to understand the scale of the discrepancies seen in reporting.

Results: From 2011/12 to 2012/13 the number of patients receiving a foot-risk assessment in Primary Care in the Borough of Southwark increased by

13% (to 84.4% of patients on the Diabetes register). In 2012/13, 17% of patients were classified as moderate or high risk, which requires regular community podiatry input. However, the actual patient activity indicated that only 8% of such patients with diabetes were seen – a gap of over half. Based on population studies of prevalence of foot risk, it would have been expected that as much as 20–30% diabetes individuals would have a moderate or high classification score. A greater disparity between hospital activity and Primary Care was seen in active diabetes foot disease. Only 0.6% of patients were coded as active, while 4% of patients were actually being seen in the two local acute Trusts' hospital foot clinics – a 6-fold difference.

Conclusion: The DMI found that only one in four patients identified to have moderate and high foot risk currently receives the desired foot follow-up. Furthermore, there was a substantial difference in active diabetes foot burden as perceived by Primary Care to that in fact seen at hospital clinics. Incorrect coding and risk stratification may have considerable impact on future commissioning of diabetes foot services as well as personnel. Improvements in coding can potentially lead to more efficient and responsive foot health services. The challenge of non-integrated care for diabetes foot patients has been met by DMI by bringing together all healthcare providers and commissioners to devise a joint strategy across sectors. This includes a locally adapted traffic light risk screening tool, structured referral forms and standardised clinical letters, now in ubiquitous use across the host CCGs. Criteria for bi-directional patient referral and discharge across community and hospital settings have now been implemented.



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Barriers to inpatient diabetic foot examination

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Background and aims: Despite NICE guidelines and Putting Feet First, only 38% of patients with diabetes receive a foot assessment in England and Wales within 24 hours of admission (National Diabetes Inpatient Audit 2013). A local audit has demonstrated an increase in foot examinations from 13 to 53% following education and introduction of a diabetic foot sticker in the acute medical admissions proforma. Our aim was to identify the major barriers to diabetic foot examination and to explain why compliance was so low.

Materials and methods: A self-administered questionnaire was designed to reflect knowledge, training, practice, confidence and barriers to diabetic foot examination. The survey was given to 110 doctors, 4 diabetic specialist nurses and 3 medical students involved in acute medical admissions.

Results: The response rate was 71%: 51% were doctors in training, 30% consultants and 9% medical students/diabetic nurses. Although 96% thought foot examination important, only 56% would do this routinely. The other 44% required a further clinical indication before they would perform an examination, such as a diabetic foot ulcer or sepsis. The greatest barriers to examination were: time constraints (86%), occlusive dressings (78%), forgetting (69%), lack of equipment (64%), lack of training (56%) and patients being too ill (41%). Foot odour was a problem for 16% and foot phobia in 11%. 69% admitted to examining less than half of their admissions and 94% examined less than half of patients' footwear. This was despite 60% highlighting poor footwear as one of the greatest risk factors for ulceration. 31% of

respondents have had no formal foot examination training, including 44% of all consultants. The preponderance (45%) of training was undertaken at medical school, the quality of which was only rated as good or excellent by 39%. Little teaching was organised on the wards (18%), in hospital (14%) or online (6%). Confidence was expressed in diagnosing vascular disease (86%) and neuropathy (84%), but staff lacked confidence with acute Charcot joints (73%), neuro-ischaemic ulcers (52%), acute osteomyelitis (49%) and neuropathic ulcers (47%). Staff thought the greatest risks for foot ulceration were: poor blood sugar control (80%), ill-fitting footwear (60%), peripheral neuropathy (54%) and peripheral vascular disease (49%). Respondents grossly overestimated the number of patients presenting with a foot ulcer leading to a new diagnosis of type 2 diabetes (median 15%). However, the prediction of amputation rates in diabetic patients with an ulcer over a 20 year period was more accurate: median 20%.

Conclusion: Despite awareness that diabetic foot examination is important, there continues to be unwillingness to perform this crucial examination unless clinical indications are present. Lack of time, barrier dressings, forgetting, equipment shortages and poor training account for most incomplete examinations. Staff lack confidence in their ability to diagnose acute Charcot joints and osteomyelitis and a significant number, especially consultants have had no formal training. Simple local interventions have the potential to significantly increase compliance with foot examinations. Regular hospital-based teaching should be made more widely available with refresher courses for consultants. Increasing resources, improving access to equipment, encouragement to remove occlusive dressings and reminders in admission booklets are all fundamental to improving the foot care of diabetic patients admitted to hospital.

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Nonvisual foot examination for people with visual impairment

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Background and aims: Because people with both diabetes and visual impairment have high risk for ulcers and amputation, prevention is a top priority. Usual care in diabetes self-management education is to teach visually impaired people to seek sighted assistance for regular foot examination, yet clinical experience suggests that this advice is seldom heeded. One possible solution is to teach use of the nonvisual senses for foot self-examination. The purpose of this pilot study was to compare the efficacy, acceptability, and feasibility of nonvisual foot examination, using the senses of touch and smell, with usual care (examination of feet by a sighted person).

Materials and methods: Fifty-two visually impaired adults with type 2 diabetes received comprehensive diabetes self-management education, with the experimental and comparison groups receiving different foot examination instructions. The experimental group was taught nonvisual foot examination, i.e., use of their own hands for a systematic tactile inspection of feet for cuts, swelling, irregularities, or warmth, and use of the nose to detect unusual odors. The comparison group received usual care instructions for visually impaired persons, i.e., to have someone with good eyesight to check their feet. All kept large print or tactile diaries of how frequently foot examination was done at home and by whom; this was reported monthly to the research team in a phone call. All had baseline podiatric evaluations and follow-up evaluations at 3 and 6 months, with extra visits if medically necessary. Participant-reported foot symptoms and podiatrist-confirmed new foot problems were recorded at each visit following the baseline evaluation. Focus groups were held after the 6 month visit.

Results: Both the experimental and comparison Groups had their feet checked a mean of about 22 times per month, with no significant difference between groups ($p = .69$). However, people in the experimental group followed the instructions they were given (i.e., checked their own feet) much more frequently ($M = 1.03$) than people in the comparison group did so (i.e., had someone else check their feet) ($M = .56$), $F(1, 45) = 41.42$, $p < .001$. This result is consistent with qualitative information gathered through the focus groups. Many in the experimental group reported checking their own feet frequently, as they were taught, with occasional backup from sighted people. In contrast, many in the comparison group reported being reluctant to follow the instructions they had been given and ask for help checking their feet. They said they checked their own feet „as best they could“, using whatever vision they had or using self-invented tactile methods, and got help when it was not too embarrassing or difficult. At podiatrist visits, people in the experimental group reported a greater number of symptoms per visit ($M =$

1.61) than people in the comparison group ($M = 1.13$), $F(2, 50) = 64.07$, $p < .001$, indicating a greater effectiveness of their home foot check methods.

Conclusion: Visually impaired people in this study who were advised to ask someone with good eyesight to check their feet did not do so regularly. Many invented their own method of self-checking in whatever way they are able. These invented methods were not as effective for detecting symptoms of foot problems at home as systematic nonvisual foot examination. Systematic methods of nonvisual foot examination should be routinely taught to visually impaired people with diabetes.

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Relationship between gait alterations and microvascular chronic complications in type 2 diabetic patients

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Background and aims: We evaluated gait alterations and their correlations with microvascular chronic complications in type 2 diabetic patients (T2DM).

Materials and methods: Thirty-six T2DM (M/F: 27/9; age: 63 ± 10 yrs; diabetes duration: 12 ± 11 yrs; BMI: 29.2 ± 5.6 Kg/m²; HbA1c $8.1 \pm 0.9\%$) attending our outpatient clinic, were divided into 3 Groups: Group 1 ($n=12$) with no diabetic neuropathy (DN) and foot ulcerations (FU); Group 2 ($n=10$) with DN and no FU; Group 3 ($n=15$) with DN and non-infected, non-ischemic FU. We analyzed biomechanical alterations of lower limbs by motion analysis system (BTS Elite Clinic, BTS Bioengineering, Milan, Italy). Spatial-temporal and kinematics data were collected through photogrammetric infrared cameras while kinetics data with two forces plates. Data were correlated with patients' microvascular chronic complications; in particular, all patients were examined with indirect and direct retinoscopy and two non-stereoscopic 45° retinal photographs for each eye. The presence and severity of retinopathy (DR) was determined according to the Eurodiab Study classification.

Results: Step Width (SW) was greater in Group 2 (240.9 ± 47.5 mm) and Group 3 (271.6 ± 41.7 mm, $p < 0.02$ vs group 1); ankle range of motion (ROM) was significantly lower in Group 3 (Group 1: $26.6 \pm 5.6^\circ$, Group 2: $26.0 \pm 4.9^\circ$, Group 3: $23.6 \pm 4.7^\circ$) (Group 3 $p < 0.05$ vs Group 1 and 2); foot ROM was significantly lower in Group 3 (Group 1: $37.2 \pm 6.6^\circ$, Group 2: $34.8 \pm 1.7^\circ$, Group 3: $30.2 \pm 1.5^\circ$) (Group 3 $p < 0.02$ vs Group 1 and 2). There was no difference in vertical ground-reaction forces. When the whole population was considered, a positive correlation was apparent between DR presence and severity and SW ($r=0.6$; $p < 0.05$) and foot ROM ($r=0.65$; $p < 0.05$).

Conclusion: Our study demonstrated a positive correlation between biomechanical alterations and presence and severity of DR in T2DM, suggesting a possible contribution of the involvement of the microvascular bed in the pathogenesis of FU.

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Increased pulse pressure and reduced baroreflex gain precede diabetic foot complication by at least 10 years

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Background and aims: This case-control retrospective study investigated whether abnormalities in arterial stiffness (estimated by increased pulse pressure: PP) and cardiovascular autonomic neuropathy (evaluated by decreased baroreflex gain: BRG) may be present far before the occurrence of diabetic foot complications and thus potentially used as predictive markers.

Materials and methods: PP and BRG were derived from a continuous (using a Finapres® device) blood pressure (BP) and heart rate (HR) during a standardised squatting test (1 min standing - 1 min squatting - 1 min standing). Measurements were collected in 20 diabetic patients with future diabetic foot complication (selected in our diabetes foot clinic, around 9 ± 4 years after initial haemodynamic assessment), in 20 patients without diabetic foot - matched for type (10 T1D/10 T2D) and duration of diabetes (mean: 24 years), age (mean: 54 years), gender (15M/5F), BMI (mean: 28 kg/m²) and HbA1c (mean: 8.2%) - and in 20 nondiabetic well matched controls. PP

was calculated by the difference between systolic BP (SBP) and diastolic BP (mmHg) during the whole 3 min test and during squatting position while BRG (msec/mmHg) was calculated by the slope of the relationship between RR intervals and SBP during the transient phase from squatting to standing.

Results: PP was higher in diabetic with (mean \pm SD: 73 ± 20 , $p < 0.0001$) and without future diabetic foot (68 ± 17 , $p = 0.001$) than in controls (50 ± 13 mmHg). During squatting, diabetic patients with later diabetic foot had much greater PP increases ($+17 \pm 12$ mmHg) than diabetic patients without diabetic foot ($+8 \pm 9$, $p = 0.009$) or controls ($+7 \pm 7$, $p = 0.003$). During squatting, increases of PP \times HR products and SBP \times HR products were more than doubled ($p < 0.05$) in patients with versus without later diabetic foot (Table). Baroreflex gain was significantly decreased in patients with later diabetic foot than in patients than in controls (1.11 ± 0.91 versus 2.46 ± 2.32 ; $p = 0.024$), but not significantly in patients free of diabetic foot (1.59 ± 1.37 ; $p = 0.163$).

Conclusion: Increased PP, mainly in squatting position, and decreased BRG were already present almost 10 years before the occurrence of a diabetic foot complication. These results are in favour of a contribution of arterial fitness and autonomic neuropathy in this complication. They also suggest that increased PP and decreased BRG may be considered as initial markers of future diabetic foot complications and thus may help selecting patients who should be more particularly supervised and advised to avoid diabetic foot complications.

	A DIABETIC FOOT	B NO DIABETIC FOOT	C NON DIABETIC CONTROL	A Vs B	A Vs C	B Vs C
N (men/women)	15/5	14/6	15/5	p	p	p
Age (years)	54.0 ± 8.4	53.8 ± 10.3	52.9 ± 5.0	0.930	0.597	0.727
Type 1/Type 2	10/10	10/10				
Diabetes duration (years)	24.3 ± 11.3	24.8 ± 10.8		0.977		
BMI (kg/m ²)	27.8 ± 6.6	27.0 ± 5.1	28.9 ± 2.9	0.649	0.519	0.156
HbA1c (%)	8.2 ± 1.2	8.1 ± 1.1		0.732		
SBP (mmHg)	139 ± 28	132 ± 23	121 ± 15	0.377	0.019	0.095
DBP (mmHg)	66 ± 16	64 ± 16	71 ± 8	0.613	0.227	0.069
Pulse pressure (mmHg)	73 ± 20	68 ± 17	50 ± 13	0.416	0.000	0.001
Heart rate (bpm)	86 ± 13	80 ± 12	85 ± 15	0.092	0.687	0.244
PP \times HR product (mmHg.min ⁻¹)	6246 ± 1954	5402 ± 1714	4176 ± 1314	0.155	0.000	0.016
SBP \times HR product (mmHg.min ⁻¹)	12026 ± 3159	10531 ± 2849	10263 ± 2351	0.124	0.053	0.747
Δ Pulse pressure (mmHg)	17 ± 12	8 ± 9	7 ± 7	0.009	0.003	0.748
APP \times HR product (mmHg.min ⁻¹)	1280 ± 1160	458 ± 896	526 ± 802	0.017	0.023	0.804
Δ SBP \times HR product (mmHg.min ⁻¹)	2126 ± 1888	907 ± 1572	728 ± 1731	0.033	0.019	0.734
Baroreflex gain (mmHg.min ⁻¹)	1.11 ± 0.91	1.59 ± 1.37	2.94 ± 0.11	0.240	0.024	0.163

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Microvascular dysfunction in diabetic Charcot neuroarthropathy

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Background and aims: Charcot neuroarthropathy is a form of diabetic foot syndrome characterized by bone and joints deformation. The pathogenesis of Charcot foot is uncertain. The aim of this study was to assess factors associated with the occurrence of Charcot neuroarthropathy in patients with diabetes.

Materials and methods: It is a case-control study. We evaluated 40 diabetic patients (31 men) with Charcot neuroarthropathy (CN-DM), median age 59 (IQR: 51-62), median disease duration 16 (9-24) years. The control group were 38 subjects with diabetes and without Charcot neuroarthropathy (DM), 29 men, median age 60 (55-62), median diabetes duration 15 (10-21) years. We assessed metabolic control of diabetes, serum C-reactive protein concentration (CRP) and cardiovascular autonomic neuropathy (ProsciCard III). We used AGE-Reader to measure skin autofluorescence (AF) associated with accumulation of advanced glycation end products that reflects long lasting metabolic control. Microvascular function was examined by laser Doppler flowmetry (PERIFLUX 5000) with thermal hyperemia and postocclusive reactive hyperemia.

Results: CN-DM patients as compared to DM subjects had better HbA1c level [7.5 (6.6 - 8.2) vs 8.2 (7.1 - 9.5)%, $p = 0.01$] and higher CRP concentration [4.8 (2.3 - 11.0) vs 1.8 (0.6 - 4.1)mg/l, $p < 0.001$]. The peak flow during thermal hyperemia (THmax) was lower in CN-DM subjects as compared to DM group

[163 (76–246) vs 254 (165–366)PU, $p=0.001$]. Additionally, we found negative correlation between THmax and CRP concentration ($R_s=-0.33$, $p=0.03$), TG concentration ($R_s=-0.46$, $p=0.002$) and skin AF ($R_s=-0.32$, $p=0.04$) and positive correlation between THmax and HDL cholesterol level ($R_s=0.58$, $p<0.001$). The cardiovascular autonomic neuropathy (CAN) prevalence was higher in CN-DM subjects [62 vs 35%, $p=0.01$].

Conclusion: Deterioration of microvascular function and autonomic system dysfunction might play a crucial role in the development of Charcot neuroarthropathy. Microvascular reactivity is related to long lasting metabolic control of diabetes and activation of inflammatory process.

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Endovascular revascularisation in type 2 diabetic patients with critical limb ischaemia: comparison of direct and indirect revascularisation according to angiosome model

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Background and aims: The angiosome model (AM) is commonly used to guide bypass and endovascular procedures in the lower limb. We evaluated whether direct or indirect revascularization, according to AM, may affect clinical outcomes in diabetic patients with critical limb ischemia (CLI) undergoing percutaneous trans-luminal angioplasty (PTA).

Materials and methods: We retrospectively evaluated 137 type 2 diabetic patients (M/F: 93/44; age: 72.9 ± 9.2 yrs; BMI: 27.7 ± 8.2 kg/m²; diabetes duration: 21.8 ± 13.4 yrs; HbA1c $8.4 \pm 1.1\%$) consecutively admitted to our Department for CLI and foot lesions (FL) who underwent successful lower limb PTA. Patients were divided in 2 groups: direct (92 pts, 67%) or indirect (45 pts, 33%) depending on whether the flow to the artery directly feeding the site of ulceration, according to AM, was successfully acquired or not. Clinical outcomes (ulcer healing rate, major amputation or death) were compared in the two groups 3 months post PTA.

Results: Healing rate was higher in direct vs indirect group (58% vs 30%, respectively, $p<0.02$). One major post-procedural amputation was necessary in the indirect group (2.2%) and none in the direct one. Mortality rate during the follow-up was 19% in direct vs 31% in indirect group ($p=NS$).

Conclusion: Our data confirm that direct revascularization of arteries supplying the FL results in greater ulcer healing rate as compared to the indirect one. Thus, AM should be considered in diabetic patients with FL whenever possible.

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Perfusion scintigraphy as a method of stem cell therapy assessment in patients with critical limb ischaemia and diabetic foot

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Background and aims: There is a lack of imaging methods for microcirculation of the lower limbs in diabetic patients. Perfusion scintigraphy with Technetium-99-methoxy-isobutyl-isonitrile (MIBI) is often clinically used for the assessment of myocardial function because of its fast uptake in mitochondrial membrane. The aim of our study was to assess perfusion scintigraphy as eligible imaging method for perfusion of calf muscles after stem cell therapy and to compare it with changes of transcutaneous oxygen pressure (TcPO₂).

Materials and methods: Perfusion scintigraphy with ^{99m}Tc-MIBI was performed before and 2 months after the autologous stem cell therapy in 19 patients with no-option critical limb ischemia (mean age 63.3 ± 9.1 years, mean diabetes duration 24.2 ± 7.4 years). TcPO₂ measurement was used as a standard method of non-invasive evaluation of limb ischemia. After application of 4 MBq/kg of ^{99m}Tc-MIBI a whole-body scan and SPECT/CT was performed; next bolus of 8 MBq/kg of ^{99m}Tc-MIBI was applied after a stress test (60times flexion and extension in ankle joint). Scintigraphic parameters such as rest count (RC) and exercising count (EC) after stress test were set up.

Ischemic ratio in rest (IRR) and after stress test (IRE) were defined as ratios of these parameters in treated to untreated (control) limb.

Results: We observed a significant improvement in IRR (from 0.44 to 0.49, $p=0.01$) as well as in IRE (from 0.47 to 0.51, $p=0.03$) after 2 months from cell treatment. The effect of stem cell therapy was confirmed by a significant increase of TcPO₂ values from baseline 12.9 ± 9 to 37.2 ± 14.8 mm Hg after 2 months ($p=0.0001$).

Conclusion: Our study showed a significant improvement in scintigraphic parameters after stem cell therapy in patients with ischemic diabetic foot which was in accordance with changes of TcPO₂. Perfusion scintigraphy could be a promising technique to objectify the microcirculation of lower limbs.

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PS 100 Management of foot ulcers

1147

A longitudinal cohort of people presenting with diabetic foot ulcers in northern England

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Background and aims: It is known that people with diabetic foot ulcers have high morbidity and mortality but there is little contemporary data 'real world' data on this group of people outside specific research trials. This population based study examines the characteristics and outcomes of people with incident diabetic foot ulcers in a longitudinal cohort of people presenting with diabetic foot ulcers in Salford in the north of England.

Materials and methods: Since 2001 data on all people with an incident diabetic foot ulcer in Salford has been recorded in an electronic clinical system as part of routine clinical practice. This database has been linked with death registration records to identify all deaths up to June 2013. Regression models were created to examine factors associated with ulcer healing, ulcer recurrence and mortality.

Results: Between 2001 and 2012 there were 8028 incident cases of diabetic foot ulcers among 2937 people. There has been no change in age at presentation (mean 68.6 years, median 70 years) or the proportion of patients that were male (59.4%). 2.4% of incident ulcers were deep enough to involve the bone, 24% were accompanied by cellulitis and 32.7% were in people with peripheral vascular disease. The proportion of people with a previously healed foot ulcer reduced from 67.2% in 2001 to 44.6% in 2012 ($p < 0.005$). Median time to healing was 79 days (IQR 21–320 days). The proportion of ulcers that healed within 90 days declined from 61.1% in 2011 to 32.1% in 2012 ($p < 0.005$). Men (OR 0.868 95% CI 0.773–0.975) and older people (OR 1.005 95% CI 1.001–1.009 per additional year) were less likely to heal within 90 days. Having peripheral vascular disease (OR 0.750, 95% CI 0.660–0.853), cellulitis (OR 0.840, 95% CI 0.736–0.960), an ulcer to the bone (OR 0.461, 95% CI 0.303–0.702) were significantly associated with a lower chance of the incident ulcer healing within 90 days whilst having a previously healed ulcer increased the chance of healing (OR 1.529, 95% CI 1.364–1.714). Of the people presenting with a foot ulcer in 2010 8.8% died within six months, 15.5% died within one year and 24.6% had died after two years. Being male (OR 1.246, 95% CI 1.002–1.549), older (OR 1.064, 95% CI 1.054–1.075 per additional year), having peripheral vascular disease (OR 2.410, 95% CI 1.949–2.978) or a rear-foot ulcer (OR 1.907, 95% CI 1.520–2.392) were associated with significantly higher mortality six months after presentation. These factors are also predictors of longer term mortality in this group.

Conclusion: The demographic characteristics of people presenting with incident diabetic foot ulcer have not changed since the turn of the century. Outcomes remain very poor with the majority of ulcers unhealed at 90 days. One in four people presenting with foot ulcers died within two years; this short term mortality is very high especially in those with co-existent peripheral vascular disease which more than doubles the risk of dying.

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Identification of factors contributing to failure of ambulatory negative pressure wound therapy in patients with diabetic foot

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Background and aims: Previous studies showed that negative pressure wound therapy (NPWT) is an effective method for the treatment of patients with diabetic foot. Indication criteria of this treatment based on patient and wound characteristics are not clear verified. The aim of our study was to assess the effect of ambulatory NPWT on diabetic foot healing and identify factors contributing to failure of this method.

Materials and methods: 148 patients with diabetic foot hospitalized in our Diabetes Department were treated by NPWT between May 2003 and October 2013. 60 patients continued with NPWT on ambulatory basis and were enrolled in the present study. The changes of dressing were done in our foot clinic 2–3 times per week. The median length of NPWT was 23 days

(7–98). The success of NPWT was defined as a complete wound healing during 6 months follow-up; the unsuccess as a premature termination of NPWT (worsening of the wound/no effect), non-healing, major amputation or intolerance of the treatment by patient during 6 month follow-up. In all patients, factors which could influence wound healing were evaluated: age, type of diabetes, duration of diabetes, diabetes control (HbA_{1c}), presence of infection, ischemia, Charcot foot, renal failure and other comorbidities, but also local factors (wound localization, size, exposed bone etc.). Uni- and multivariate analyses were used to identification of factors contributing to failure of ambulatory NPWT.

Results: During follow-up period, 47/60 (78.3%) patients were completely healed after ambulatory NPWT, in 13/60 (21.7%) patients was not NPWT successful. In univariate analysis, the unsuccess of NPWT was influenced especially by poor diabetes control (HbA_{1c} in unsuccess vs. success; 77.2 ± 19 vs. 62.5 ± 18.6 mmol/mol; $p = 0.01$). Potential factors of NPWT failure should be also exposed bone in the wound (76.9% of patients with unsuccess vs. 46.8% with success; $p = 0.1$) and haemodialysis (23.1% vs. 4.2%; $p = 0.1$ resp.). There were no significant differences in other factors assessed in univariate analysis. Logistic regression showed that HbA_{1c} (OR 1.05; 95% CI 1.01–1.09; $p = 0.01$), haemodialysis (18; 1.6–208.3; $p = 0.02$) and exposed bone (7.8; 1.3–48.1; $p = 0.03$) were significant factors for failure of ambulatory NPWT, other followed factors were not significant.

Conclusion: Ambulatory NPWT was effective in majority of patients, but poor diabetes control, haemodialysis or exposed bone in the wound may contribute to the failure of this method. These results showed that patients with diabetic foot treated by ambulatory NPWT require precise follow-up focused on diabetes control and it is necessary to consider the indication in patients on haemodialysis and with exposed bone in the wound.

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Arterial stiffness influence outcome of chronic diabetic foot ulcers in patients with type 2 diabetes

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Background and aims: Chronic diabetic foot ulcers are estimated to occur in 15% of all patients with diabetes and precede 84% of all diabetes-related lower-leg amputations. The pathophysiology of chronic diabetic foot ulcers still incompletely understood, but both micro- and macroangiopathy strongly contribute to the development and delayed healing of diabetic wounds. Pulse wave velocity (PWV) is a method to estimate arterial stiffness and predicts cardiovascular morbidity and mortality. Several studies demonstrated that pulse wave velocity is a sensitive predictor for peripheral artery disease and cerebrovascular disease among diabetic patients. The objective of this study was to determine whether arterial stiffness index [brachial-ankle pulse wave velocity (baPWV)] can influence outcome of chronic diabetic foot ulcers in patients with type 2 diabetes.

Materials and methods: We recruited a total of 102 type 2 diabetic patients (63 men and 39 women) with chronic nonhealing diabetic foot ulcers with Wagner grade 1 or 2 ulcers that are ≥ 2 cm in largest diameter at diagnosis for more than 1-month duration. The age was 65.2 ± 16.4 years, and the diabetes duration was 18.4 ± 16.0 years. Anthropometric, clinical, and laboratory data were measured. All patients were seen bi-weekly for debridement, offloading, and other treatments during the initial 8 weeks. The PWV was measured between the brachial and ankle regions (baPWV), and the baPWV was measured in all patients using a waveform analyzer.

Results: At 8 weeks, 49 of the 102 ulcers had completely healed. The patients were assigned into healed group ($n = 49$) or unhealed group ($n = 53$) according to clinical outcome of ulcer healing at 8 weeks. There were not significantly different in age, duration of diabetes, HbA_{1c} , or initial wound size in diameter of the ulcer between the healed and unhealed groups. The healing time of foot ulcers in healed group was 5.4 ± 2.8 weeks (range 2.8–8.0). The baPWV was significantly ($P < 0.05$) higher in the unhealed group (1773 ± 279 cm/s) as compared with the healed group (1547 ± 340 cm/s). ABI or TBI was not significantly different between unhealed and healed group. Age ($r = 0.463$; $p < 0.01$), duration of diabetes ($r = 0.425$; $p < 0.05$), ankle-brachial index (ABI) ($r = -0.349$; $p < 0.05$) and toe-brachial index (TBI) ($r = -0.264$; $p < 0.05$) were significantly correlated with baPWV. But BMI, lipid profiles, HbA_{1c} , and systolic and diastolic BP were not correlated with baPWV. Univariate analysis revealed that baPWV was significantly correlated with healing rate of diabetic ulcers ($r = 0.321$, $p < 0.05$) and duration of diabetes ($r = 0.412$, $p < 0.01$).

Conclusion: These findings indicate that baPWV is closely associated with the healing time of diabetic ulcers. We suggest that the measurement of systemic arterial stiffness may help to identify ulcers at risk of poor healing in chronic diabetic foot ulcers in patients with type 2 diabetes.

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The association between Neuropad testing with foot ulceration in diabetes

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Background and aims: Foot ulceration in patients with diabetes is a serious complication associated with increased morbidity, mortality and healthcare cost and is the main cause of amputation. The prevalence of foot ulcers is 4% to 10%, and the annual population-based incidence is 1.0% to 4.1%. Prevention of foot ulceration and consecutively amputation begins with identification of those at risk. Well-established risk factors for foot ulceration are previous foot ulceration and lower extremity amputation, long duration of diabetes, poor glycemic control, severity of diabetic neuropathy, foot deformities and visual impairment. Cross-sectional data have shown that dryness of the skin of the feet assessed by either sympathetic skin response or Neuropad testing has been associated with foot ulceration in patients with diabetes. In addition, Neuropad testing has a high performance for the diagnosis of diabetic peripheral neuropathy and is proper for self-testing. The aim of the present prospective multicenter study was to examine the association between Neuropad testing with foot ulceration in patients with diabetes.

Materials and methods: A total of 308 patients with diabetes (155 females and 153 males; 280 with type 2 diabetes; mean age 62.8 ± 11.3 years; mean diabetes duration 12.4 ± 9.7 years) with no history of foot ulceration were recruited in the study from the year 2005 until the year 2012. At baseline participants were evaluated for neuropathy status using the neuropathy disability score (NDS). Patients with NDS 0–2 were considered as having no neuropathy, those with NDS 3–5 as having mild neuropathy and those with NDS ≥ 6 as having severe neuropathy. In addition Neuropad testing was performed and the results were evaluated as normal or abnormal based on complete colour change of the test after 10 min of application.

Results: At baseline, 148 patients (48.1%) did not have neuropathy, 82 (26.6%) had mild neuropathy and 78 (25.3%) had severe neuropathy. Neuropad testing was normal in 128 (41.6%) and abnormal in 180 (58.4%) patients. The mean follow-up was 5.5 ± 2.5 years. During this time, 55 (17.9%) patients developed foot ulcers. At baseline, patients who developed foot ulcers were older ($p=0.03$) and had longer diabetes duration ($p=0.01$). After adjustment for age, gender and duration of diabetes, abnormal Neuropad testing at baseline was associated with increased odds (OR, 95% confidence intervals) for foot ulceration [4.2 (1.8–9.8)]. Similarly, the adjusted OR of NDS ≥ 6 vs. NDS < 6 for foot ulceration was 8.5 (3.3–21.7). The OR for foot ulceration was not increased significantly ($p=0.09$) in those having mild neuropathy (NDS 3–5) vs. those having no neuropathy.

Conclusion: These prospective demonstrate that abnormal Neuropad testing is associated with a 4-fold higher risk for foot ulceration. Neuropad testing can be included in the screening tests for the prevention of foot ulceration in patients with diabetes.

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1151

Measurement of TcPO₂, toe pressures and laser Doppler flow reveals hitherto undiagnosed subclinical ischaemia in patients with palpable pedal pulses and foot ulceration

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Background and aims: Patients presenting with a diabetic foot ulcer should be assessed and treated urgently as a “foot attack”. However, their clinical vas-

cular assessment is often limited to palpation of pulses and measurement of Ankle Brachial Pressure Index (ABPI). The aim of this study was to investigate the contribution of a full assessment of the macro- and microcirculation in patients with foot ulceration.

Materials and methods: Consecutive patients with ulceration in one or both limbs underwent measurement of ABPI, Toe Blood Pressure Index (TBPI), TcPO₂ in both feet, at supine and at 30° elevation and simultaneously in forearm, and forefoot laser Doppler flowmetry, in perfusion units (PU), at baseline and with heat provocation. All measurements were carried out within 35min. Results are reported as mean \pm SD.

Results: There were 53 limbs in 27 patients, age 57 ± 9.8 yrs, 93% males, 86% with type 2 diabetes and 66% of limbs were ulcerated; 40 limbs had palpable pedal pulses (Group 1) and 13 limbs had a known diagnosis of ischaemia (Group 2). Unexpectedly 58% of group 1 had evidence of ischaemia (we called Group 1a) as denoted by a low TBPI of ≤ 0.7 (mean 0.5 ± 0.1), compared with the remaining patients (Group 1b), with normal TBPI (mean 1.0 ± 0.2) [$p=0.001$]. Doppler flow increment on heating was less in Group 1a, 12 ± 9 to 52 PU compared with Group 1b, 12 ± 4 to 68 PU [$p=0.03$]. However, there was no significant difference between Group 1a and 1b as regards ABPI, 1.1 ± 0.2 vs 1.2 ± 0.2 [$p=0.053$] nor TcPO₂ at supine, 52 ± 11 vs 55 ± 8 mmHg [$p=0.55$]. However, foot elevation identified 10% in group 1b with a ≥ 15 mmHg drop in TcPO₂ despite their normal TBPI. As expected, in group 2 (known diagnosis of ischaemia), mean TBPI was 0.5 ± 0.3 , baseline TcPO₂ 39 ± 20 mmHg. There was no difference in proportion of ulcers across all groups, $p=0.79$.

Conclusion: We have identified a subgroup of patients with palpable pulses that have a low TBPI and a reduced heating response to Doppler flowmetry but a preserved TcPO₂ at baseline. This subgroup is distinct both from patients with palpable pulses and normal TBPI who have a normal TcPO₂ and heat response and also distinct from patients with known ischaemia in whom all these parameters are abnormal. This demonstrates that it is essential for all patients to have a thorough examination of both macro- and micro circulation on presentation to the multidisciplinary team with a foot attack.

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Extent of disagreement and difference between tissue and swab samples from infected diabetic foot ulcers: the CODIFI Study

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Background and aims: A common complication of diabetic foot ulceration is a wound infection. Treatment with antibiotics is usually started immediately as spreading infection can lead to amputation. At the same time, however, a sample is usually taken for microbiologists to advice on the types of organisms in the wound, and what antibiotics they are sensitive to. Clinical practice guidelines state that tissue samples are the best way to collect samples of wound bacteria for the microbiological analysis, but wound swabs are commonly used. The CODIFI (concordance in diabetic foot infection) study set out to determine whether the results from wound swab and tissue samples taken from the same ulcer ‘agree’ with each other.

Materials and methods: Consenting patients with an infected diabetic foot ulcer had both swab and tissue samples taken from their ulcer for microbiological analysis (plating and culture). Agreement was assessed between techniques based upon the reported presence of ‘likely pathogens’. We reported overall prevalence, Kappa statistic, and McNemar’s test to investigate patterns of disagreement

Results: We recruited 401 patients from 25 centres (2011–2013). They had a median age of 63 years; 79% were male; 85.5% had type 2 diabetes; 27.5% presented with a recurrent ulcer; and 45.5% had a neuro-ischaemic ulcer, 50.5% neuropathic ulceration, and 3.5% ischaemic ulceration. Swab and tissue reports were available for 395 patients. We found many ‘likely pathogens’ - the most prevalent were Gram Positive Cocci (70.4%), Gram Negative Bacilli (36.5%), Staphylococcus Aureus (35.7%), Anaerobic Cocci (20.5%), Coagulase-Negative Staphylococcus (12.2%), Gram Positive Bacilli (11.1%), Streptococcus (16.7%), Enterococcus (14.9%), Corynebacterium (9.4%), Pseudomonas (8.6%), and Methicillin-resistant S. Aureus (MRSA, 8.1%). With the exception of Staphylococcus Aureus, MRSA and Pseudomonas (for which identical discordance was observed), each isolate was reported in significantly more tissue samples than the swab (p -value < 0.05) by between 3.3% (Streptococcus) and 13.4% (Gram Positive Cocci).

Conclusion: Overall, significantly more isolates are reported from tissue samples than swab samples in patients with infected diabetic foot ulcers. This has potential implications for choice of sampling technique in practice.

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Conservative management of neuropathic heel ulceration with calcaneal osteomyelitis and avulsion fracture in a cohort with diabetic foot disease

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Background and aims: The complex of neuropathic heel ulceration, calcaneal osteomyelitis (OM) and calcaneal fracture in people with diabetes is rare and challenging to manage, especially when complicated by tendon avulsion. We describe 4 patients in whom a conservative non-operative approach resulted in limb salvage.

Materials and methods: All patients were managed in the multidisciplinary diabetic foot clinic. Specialist input was provided by diabetologists, podiatrists, radiologists, microbiologist, vascular surgeons, orthopaedic surgeons, neurologist and orthotists. Multidisciplinary management included, where appropriate, antibiotics, wound debridement, vascular intervention, vacuum pump therapy and heel offloading. Patients were closely monitored clinically and radiologically for wound healing, resolution of OM and preservation of ankle function.

Results: Four patients were included. Their demographics and management are summarised in table 1. Two subjects had multidrug resistant organisms identified. Resolution of complex heel ulceration with calcaneal fracture and tendon avulsion is ongoing with 9 (6-11) months of follow up to date and a similar duration of antibiotics (9 (8-9) months). Vascular surgical intervention was required in 2 out of 4 subjects and adjunctive vacuum pump dressings were used in all subjects. Ankle function was at least partially preserved in all subjects.

Conclusion: This is the first description of a cohort of patients with diabetes, heel ulceration and calcaneal OM complicated by an avulsion fracture managed conservatively. Vacuum pump therapy was applied, not only to soft tissue, but also to bone, with progressive wound healing. Despite tendon avulsion, 3 of 4 patients have preserved ankle function and there were no minor or major amputations. Conservative management so far has been successful despite the presence of multiple resistant organisms in 2 of the 4 subjects. This complex can occur following an ulcer or trauma, requires prolonged therapy and has serious physical, psychosocial and biomechanical implications. There is no consensus on conservative or surgical management and functional limb salvage is often unachievable resulting in a major amputation. Whilst this is a small case series, this challenging diabetes foot problem is too rare to examine by randomised controlled trial. These 4 cases, managed successfully by a highly skilled multidisciplinary team provide a template for management.

Patient Characteristics	Patient 1	Patient 2	Patient 3	Patient 4
Gender, Age	Male, 54	Male, 65	Female, 50	Female, 35
HbA1c	49	83	111	65
DM type	Type 2	Type 2	Type 2	Type 1
Diabetes duration (years)	7	35	17	22
CKD	Stage 4	No	Stage 3	Renal transplant
Peripheral neuropathy	Yes	Yes	Yes	Yes
Follow-up (months)	11	9	9	6
History of trauma	No	Yes	No	No
Lesion	Right Calcaneal ulcer	Right Calcaneal ulcer	Right Calcaneal ulcer	Left Calcaneal ulcer
Organism	MRSA, multidrug (MDR) Pseudomonas	Staphylococcus	Coiforms, Enterococcus, Diptheroids E coli	MRSA, MDR pseudomonas, E. coli, coiforms
MRI imaging	Calcaneal OM, avulsion fracture	Calcaneal OM, avulsion fracture	Calcaneal OM with avulsion fracture	Calcaneal OM, achilles tendon rupture
Antibiotic duration (months)	9	9	9	8
Antibiotics given during the course of treatment	Rifampicin Doxycycline Vancomycin Ceftazidime Meropenem	Rifampicin Trimethoprim Tazodin Meropenem	Augmentin Ciprofloxacin Meropenem	Augmentin Ciprofloxacin fusidic acid Vancomycin Tigecycline
PVD Details	Posterior tibial artery & peroneal angioplasty 2011 & superficial femoral artery angioplasty 2013	Non-obstructive distal atherosclerosis	Non-obstructive distal atherosclerosis	Left superficial femoral artery angioplasty & stent
Offloading method	Pressure Relief Ankle Foot Orthosis (PRAFO)	PRAFO Volar slab	Split removable cast & PRAFO	PRAFO
Debridement method	Surgical & sharp	Surgical & sharp Partial calceotomy	Surgical & sharp	Sharp
Vac pump used	To soft tissue	To bone	To soft tissue	To bone
Wound progress	Almost healed	Almost healed	Almost healed	Slow healing
Ankle function preserved	Complete	Complete	Complete	Partial

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The efficacy of removable offloading devices to heal plantar foot ulcers in diabetic patients

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Background and aims: Adequate offloading is required for healing neuropathic plantar foot ulcers in patients with diabetes. Guidelines recommend non-removable offloading as primary treatment for these ulcers. However, in clinical practice removable offloading devices are more commonly used, mainly for practical reasons. The aim of this study was to assess the efficacy of three commonly used removable offloading devices on success in healing of plantar neuropathic foot ulcers in diabetes.

Materials and methods: A total 60 diabetic patients (48 male, mean age 62.5 years, 87% type 2) with a neuropathic non-infected, non-ischemic plantar foot ulcer were randomized to one of three treatment modalities: a bivalved total contact cast (BTCC), a Mabal Cast shoe (MABAL), or a forefoot offloading shoe (FOS). Patients were followed until healing or until 20 weeks, whichever came first. Primary outcomes were percentage healing in 12 and 20 weeks time.

Results: Foot ulcers were located at the hallux (n=24), first metatarsal head (n=21), other metatarsal heads (n=13) and toes (n=2). Forty-nine of the 60 foot ulcers were classified as small (< 2 cm²), 11 as large (> 2 cm²). Healing rates within 12 and 20 weeks according to intention-to-treat were 60% and 63% for BTCC, 60% and 83% for MABAL, and 70% and 80% for FOS (non-significant between conditions, p=0.703 and p=0.305 for 12 and 20 weeks respectively). Per protocol healing rates were 57% and 69% for BTCC, 67% and 87% for MABAL, and 77% and 88% for FOS (non-significant between conditions, p=0.519 and p=0.374 for 12 and 20 weeks respectively).

Conclusion: Healing rates were not significantly different between the three removable devices, but the off-the-shelf FOS condition showed higher healing percentages than the two casting conditions at 12 weeks. Healing rates for the BTCC were substantially lower than previously found for non-removable total contact casts (~90% healing rates), while healing rates for the other two devices are comparable for those previously found for similar removable offloading devices. A lack of forced adherence is suggested to be an explanatory factor in lower healing rates found compared to non-removable offloading, which stresses the importance of continuous pressure relief in ulcer healing.

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PS 101 Diabetic eye disease

1155

Ophthalmic biomarkers of diabetic neuropathy: does neural dysfunction precede vascular changes?

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Background and aims: To evaluate structural- functional changes in the neural level of retina and cornea in type 1 diabetic (T1DM) patients without clinical evidence of retinopathy.

Materials and methods: 30 T1DM patients (Age: 48 ± 2 yrs; Duration diabetes: 28 ± 3 yrs, HbA1c: 7.6 ± 0.5 %) with no evidence of retinopathy and 15 aged matched healthy controls underwent detailed neurological (including Neuropathy Deficit Score (NDS)) and ophthalmic assessment including measurements of global and sectorial retinal nerve fibre layer (RNFL) thickness using Spectral Domain OCT (SD-OCT) (SPECTRALIS, Heidelberg Engineering) and retinal ganglion layer function (Flicker-Defined-Form (FDF)) using Heidelberg High Edge Perimetry (HEP). Corneal nerve fibre length (CNFL) and density (CNFD) were assessed using a corneal confocal microscope (Heidelberg HRT III) and corneal sensitivity was quantified using non- contact corneal aesthesiometer (NCCA).

Results: There was a significant reduction in the global and sectorial RNFL ($P=0.01$) in diabetic patients compared to controls which correlated with the severity of peripheral neuropathy measured by NDS ($r = -0.403$, $P=0.01$). FDF ($P=0.01$), CNFD ($P=0.01$), CNFL ($P=0.01$) and corneal sensitivity ($P=0.01$) were significantly reduced in T1DM compared to control subjects and they were significantly correlated with NDS. A greater proportion of T1DM patients showed abnormal retinal structure (RNFL-61%) and function (FDF-52%) compared to corneal structure (CNFD-32%) and function (NCCA-20%).

Conclusion: Changes at the level of nerve fibre layers were observed in both the retina and cornea. However, the prevalence of abnormality was higher at the retina but the severity was more pronounced in the cornea especially in those patients without neuropathy. The results of this preliminary study suggest that structural changes in the cornea (Peripheral nerves) and in the retina (central nervous system) may occur in parallel and are correlated with functional changes.

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Early detection of neurodegenerative changes in diabetes: systematic review and meta-analysis

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Background and aims: The onset of diabetic neuropathy is difficult to detect by electrophysiology. The earliest damage occurs in the small nerve fibers. These fibers can be evaluated by thermal and pain perception, which have limited reproducibility, and by skin biopsy, which is invasive. A more reliable and feasible approach could be the imaging and quantification of neurodegenerative changes in the diabetic eye. Aims: To systematically assess ocular neurodegenerative changes in adults with type 1 or type 2 diabetes, their relationship with diabetic retinopathy and neuropathy, and whether these changes are already present in an early stage of diabetes.

Materials and methods: This meta-analysis was conducted according to the PRISMA statement and the Cochrane collaboration. The databases searched were MEDLINE, the Cochrane Controlled Trials Register and EMBASE (1968-2013). Language restrictions were English, French, German or Dutch. Only studies of neurodegenerative changes in the retina, the optic nerve head or the cornea in adults (>18 years) with type 1 or type 2 diabetes mellitus were included. One author reviewed studies for inclusion, quality and risk of bias. The second author checked the extracted data.

Results: 41 out of 4051 articles were included (3043 diabetic and 2021 non-diabetic individuals). 35 neurodegenerative parameters were analyzed. Compared with non-diabetic individuals, diabetic patients with any level of severity of diabetic retinopathy, had a mean reduction in thickness of at least 2 μ m in 5 retinal layers: the mean ganglion cell/inner plexiform layer in the peripheral area and the inner plexiform layer, the ganglion cell layer, the mean

outer nuclear layer/inner segments and the mean ganglion cell/inner plexiform layer thickness in the pericentral area of the macula. In the optic nerve head, the highest reduction was observed for the superior retinal nerve fiber layer thickness (superior RNFL: -7.98μ m, 95%CI -12.46 to -3.50). In the cornea, the nerve branch density (NBD) and the nerve fiber density (NFD) were lower by $12.80/\text{mm}^2$ and $7.45/\text{mm}^2$, respectively (95% CI -14.56 to -11.04 and -9.88 to -5.01). In patients without diabetic retinopathy the pericentral outer nuclear layer and inner segment were reduced by 2.45μ m (95% CI -4.76 to -0.14). In the optic nerve head the superior and mean RNFL thickness were decreased by 3.24 and 2.37μ m respectively (95% CI -7.31 to 0.83 and -7.32 to 2.59). In the cornea the NBD ($-7.77/\text{mm}^2$, 95% CI -12.20 to -3.34) and the NFD ($-3.57/\text{mm}^2$, 95% CI -7.12 to -0.02) were lower. In patients with any severity of diabetic polyneuropathy, the NBD and the NFD of the cornea were decreased by $20.84/\text{mm}^2$ (95% CI -23.92 to -17.77) and $16.32/\text{mm}^2$ (95% CI -19.26 to -13.37). Ocular changes were already present in patients without diabetic polyneuropathy (NBD: $-9.99/\text{mm}^2$, 95% CI -15.26 to -4.72 ; NFD: $-7.80/\text{mm}^2$, 95% CI -12.32 to -3.28).

Conclusion: This systematic review establishes a correlation between ocular neurodegenerative changes and diabetes. Structural changes are already present in diabetic individuals without diabetic retinopathy or neuropathy. Imaging and quantification of ocular neuronal tissues gives the opportunity to study diabetic neuronal changes and to early detect diabetic neuropathy.

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An evaluation of different methods of normalising fovea thickness measurements from optical coherence tomography instruments

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Background and aims: The assessment of macular thickness by optical coherence tomography (OCT) is routinely used in clinical practice. However, interpretation is hampered by the lack of a universal clinical scale, partly due to variations in the segmental algorithms between devices. For example the Stratus, Cirrus and Topcon OCTs all measure thickness to different depths: inner/outer photoreceptors interface, mid and inner limit of the retinal pigment epithelium respectively; resulting in a systematic difference when comparing measurements between devices. Previous work have proposed the logarithmic transformation of normalised data, and then utilising step changes to define clinically significant changes. Using this approach fovea data has been normalised to fovea thickness as measured by Stratus (200 μ m) and mid-range normal fovea thickness as measured by spectral domain (SD) OCTs (250 μ m) in healthy eyes. Utilising these normalisation values may still result in a systematic difference when comparing measurements from different devices. Alternative approaches are to either customise the normalisation factor to each device or to normalise data to a 'gold standard' before log transformation. As part of the SUMMIT consortium this study aims to explore different normalisation procedures for combining data from different OCT devices.

Materials and methods: 43 individuals were recruited (11 controls, 32 with diabetes: 14, 15, 5 with no retinopathy, non- and proliferative retinopathy respectively). Macular thickness was assessed in all participants on both Cirrus and Topcon-1000 OCT devices (512x128 scanning protocol). Raw data comparisons demonstrated that, as expected, Topcon measurements were consistently lower than with the Cirrus in all quadrants (mean (SD) difference: $7.9(0.5)\%$, $p < 0.001$ paired t-tests). Fovea data was log10 transformed following normalisation by expected normal fovea thickness by 1) Stratus (200 μ m); 2) mid-range SD-OCTs (250 μ m); 3) customised to device (based on literature: Cirrus: 270 μ m, Topcon: 230 μ m); 4) Topcon measurements normalised to Cirrus based on raw data (8% increase). Transformed data was then examined using Bland-Altman plots and limits of agreement.

Results: Normalising Topcon fovea measurements to Cirrus measurements successfully eliminated the systematic difference between devices. The limits of agreement for this data before and after log transformation are -16 to 17μ m and -0.025 to 0.027 respectively. Similar trends were observed in all macular regions. A systematic difference was observed with the other fovea normalisation approaches.

Conclusion: This study suggests that systematic differences in macular thickness measured by different OCT devices may be eliminated; however, limits of agreement and methods of normalisation need to be considered.

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Longitudinal association of retinopathy and glucose metabolism in a Japanese populationT. Nakagami¹, K. Takahashi¹, C. Suto², A. Hirose¹, Y. Uchigata¹;¹Diabetes Center, Tokyo Women's Medical University, Tokyo,²Department of Ophthalmology, Saitama-ken Saiseikai Kurihashi Hospital, Kuki City, Japan.

Background and aims: The epidemiological data for diabetic retinopathy have been extensively analyzed, however, limited studies have assessed the development of retinopathy across different glucose levels ranging from normal glucose tolerance to diabetes. Some studies have reported that retinopathy increased the risk of vascular disease and incident diabetes. Here, we assess the 6-year incidence for retinopathy across different glucose categories and the association of retinal signs detected at baseline with development of diabetes in a Japanese population.

Materials and methods: Subjects were 3,580 among 5,200 health check-up examinees (2,353 men, mean age: 51±8 years old) who underwent the test of non-mydriatic 45° digital fundus photography. The retinal and glucose status were followed up for 6 years in 181 and 3,399 individuals, with and without retinopathy, respectively, at baseline (mean follow up: 5.7 years). Individuals were classified into five categories according to fasting plasma glucose (FPG) (<90, 90–97, 98–125, ≥126 mg/dL, known diabetes) or HbA1c (<5.38, 5.38–5.67, 5.68–6.49, ≥6.50%, known diabetes) at baseline, and the incidence of retinopathy was calculated in each category. Multivariable logistic regression analysis to examine the effects of the presence of retinopathy as well as FPG or HbA1c at baseline on incident diabetes was performed.

Results: 1) Of 3,194 non-diabetes and 287 diabetes without retinopathy at baseline, 1,926 were followed (follow-up rate: 55.3%). The incidence of retinopathy was 2.5% (48/1,926) during 6 years and increased with deteriorating glucose category evaluated by HbA1c ($P=0.005$): 1.5, 2.3, 3.0, 4.4, and 6.6%, but not by FPG ($p=0.08$). The significant positive relationship between incident retinopathy and HbA1c category remained after adjusting for age ($p=0.021$). 2) The incidence of diabetes during 6 years was 4.4% (91/2,050) and 12.0% (10/83), respectively, among those with and without retinopathy at baseline. Multivariable logistic regression analysis showed that retinopathy at baseline as well as FPG or HbA1c, BMI, and SBP at baseline were significantly and independently associated with incident diabetes during 6 years. Adjusted odds ratios associated with the presence of retinopathy at baseline for incident diabetes during 6 years were 2.43 (95% Confidence Intervals: 1.05–5.65) and 2.95 (1.32–6.51) in the models including HbA1c and FPG at baseline, respectively, as independent variables.

Conclusion: The incidence of retinopathy during 6 years of follow-up was 2.5% in a Japanese population, and it increased with deterioration of glucose categories based on HbA1c. Non-diabetic individuals with retinopathy at baseline had a more than 2-fold higher risk of developing diabetes within 6 years than those without retinopathy. Our result suggests the importance of retinal signs as a preclinical marker of diabetes risk in the future.

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Diabetic retinopathy prevalence in patients with type 2 diabetes not performing regular eye examination

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Background and aims: Regular eye examination is crucial in preventing diabetic retinopathy. As recommended, patients with type 2 diabetes should have an initial eye examination by an ophthalmologist at or shortly after diabetes diagnosis, and then have it performed on annual basis. However, a significant number of patients do not comply with this recommendations and fail to perform regular eye examination. The aim of this study was to evaluate the frequency of retinal abnormalities in patients with type 2 diabetes who have not had eye exam performed for at least three years.

Materials and methods: The study group consisted of 674 patients with type 2 diabetes for more than 5 years and had not their eyes examined for at least three years, which meant missing two annual eye exams. All patients underwent eye fundus examination by an experienced ophthalmologist. Moreover, 103 patients completed a questionnaire containing questions related to the reasons for not reporting for ophthalmologic examinations.

Results: The reasons for not reporting to the regular eye exams was the lack of awareness of eye examination importance (37%), lack of time (26%), lack of referral letter (21%). There were no differences between the treatment groups in the incidence of hypertension or smoking. 303 (45%) patients were treated with insulin only, while 212 (31.4%) were taking insulin and oral drugs.

Conclusion: Diabetic retinopathy was found in a significant number of the patients who failed to show up for their annual eye exam, with the duration of diabetes as the main risk factor. Regrettably, despite long duration of the disease a significant proportion of patients are not aware of the need for regular eye examination. This finding calls for more intensive education regarding eye complications in the wide range of type 2 diabetes patients.

	No apparent retinopathy	Mild NPDR	Retinopathy Moderate/severe NPDR	proliferative	
Patients: 674 (100%)	421 (62.5%)	218 (32.3%)	13 (2%)	22 (3.2%)	NS
Age [yrs]	61.4±10.5	64.3±9.1	64.3±4.2	61.7±3.1	NS
Weight [kg]	93.6±22.2	88.4±18.5	83±20.22	90.8±15.5	NS
Height [cm]	169.2±7.2	167.6±8.8	166.8±7.2	166.8±11.5	NS
Diabetes duration [yrs]	12.8±6.1	15.4±7.1	14.7±8.6	22±11.5	p<0.001
Daily insulin dose [U]	60.4±35.6	60.3±31.9	49.6±17.5	55±36.4	NS
HbA1c [%]	9.4±1.6	9.2±1.3	8.8±1.6	9.4±2.8	NS
Total cholesterol [mg/dl]	191.5±46.5	175.9±54.1	204±42.4	167.2±23.5	NS
Triglycerides [mg/dl]	206.9±206.8	155.2±73.9	237.5±201.1	144±64.7	NS
HDL cholesterol [mg/dl]	41.4±11.1	40.3±12.6	47.5±21.9	36.75±11	NS
LDL cholesterol [mg/dl]	114.4±38.2	108±48.1	129.7±34.8	101.7±26.7	NS

NPDR – non-proliferative diabetic retinopathy

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Validation of a simple stratification algorithm for progression to sight threatening diabetic retinopathy

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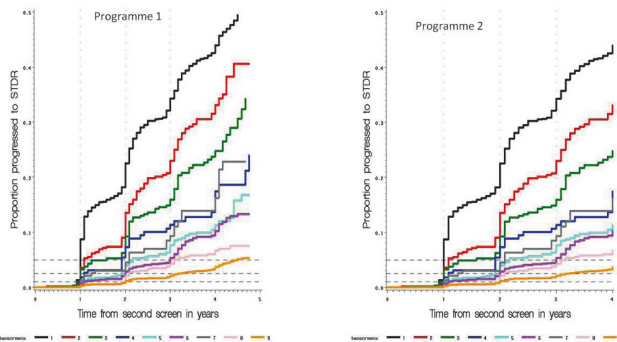
Background and aims: Diabetic retinopathy (DR) is a microvascular complication of diabetes and can lead to vision loss and blindness. Annual screening with 2-field digital retinal imaging after mydriasis is recommended by the UK National Screening Committee. This is becoming difficult to achieve because of resource limitations and increasing numbers of people with diabetes. We have previously published a risk stratification model based on the results of 2 consecutive screening episodes. We know that grading protocols differ between screening programmes and rates of DR are affected by duration of diabetes, ethnicity, deprivation and diabetic control, hence validation of the model is required in screening programmes to assess whether the stratification tool could be used more widely. Here we aim to validate this stratification in independent samples.

Materials and methods: Data were obtained from 2 English screening programmes. Patients free of sight threatening DR (STDR) at each of two successive screening episodes were categorised by the presence of DR in neither, one or both eyes at each screening. Using this risk categorisation the proportion with progression to STDR in each of 9 groups was calculated using Kaplan Meier estimation.

Results: The programmes had 24,509 and 32,987 people respectively who had been screened at least three times with no STDR at either of the first two episodes. There were few non White Caucasian patients in the first programme but 30% of those in the second programme were of African or Afro-Caribbean ethnicity. In the first programme by 4 years from the second baseline screening 9.5% of patients had been found to have STDR. In the highest risk group of 1838 people (7.5% of total) (those with mild NPDR in both eyes on each of two occasions) 44.0% had been found to have STDR by 4 years, in the middle risk group (n=1055, 4.3%) (those with mild NPDR in one eyes on each of two occasions) 11.3% had progressed and in the lowest risk group (n=15826 64.6%) (those with no DR in both eyes on each of two occasions) 3.7% had progressed by 4 years. The c-statistic was 0.76. In the second programme, by 4 years from the second baseline screening 12.1% of patients had been found to have STDR. In the highest risk group of 1669 people (6.5% of total) (those with mild NPDR in both eyes on each of two occasions) 54.0% had been found to have STDR by 4 years, in the middle risk group (n=1201, 4.7%) (those with mild NPDR in one eyes on each of two occasions) 19.5% had progressed and in the lowest risk group (n=16570 64.5%) (those with no DR in both eyes on each of two occasions) 4.5% had progressed by 4 years. The c-statistic was 0.80.

Conclusion: Within each of these two programmes the stratification enables categorisation of patients into those with very low of progression to STDR and those at high risk. This information could be used to inform decisions about screening intervals.

Time to referable DR from background or no DR



Supported by: NIHR HTA

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Male sex, blood pressure and heart rate are associated with the risk of diabetic retinopathy in normoalbuminuric type 1 patients

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Background and aims: The pathogenesis and the onset of diabetic retinopathy (DR) are still insufficiently understood. It is presumed that retinopathy and nephropathy occur simultaneously and that the severity of retinopathy equals that of renal disease in diabetes. The aim of this study was to assess the prevalence of retinopathy in normoalbuminuric type 1 diabetic patients and evaluate the risk factors for its development and progression in these patients.

Materials and methods: A total of 223 normoalbuminuric type 1 diabetic patients (116 male / 107 female) with normal renal function (glomerular filtration rate ≥ 60 ml/min/1.73m²) were included in this study and followed for 48 months. Basic and anthropometric parameters assessed were sex, age, diabetes duration and body mass index (BMI). Glycated haemoglobin (HbA1c), HDL and LDL cholesterol, triglycerides and serum creatinine were determined using routine laboratory methods. Glomerular filtration rate (GFR) was estimated using CKD-EPI formula. Urinary albumin excretion rate (UAE) was measured from at least two 24-hr urine samples and determined as the mean of 24-hr urine collections. Blood pressure was measured with a mercury sphygmomanometer and resting heart rate using a standard 12-lead ECG, after a 10-minute resting period. Ophthalmologic examination included binocular indirect slit lamp fundoscopy and color fundus photography after mydriasis of two fields (macular field, disc/nasal field) of both eyes according to the EURODIAB retinal photography methodology. Possible risk factors for the development and progression of DR were examined in backward stepwise Cox's multiple regression analysis.

Results: Patients were 38.2 ± 10.3 years old with mean diabetes duration of 17.2 ± 9.1 years. Mean/median values of BMI ($24 (18 - 37)$ kg/m²), HDL cholesterol (1.7 ± 0.4 mmol/L), triglycerides (1.02 ± 0.6 mmol/L), systolic ($120 (80 - 180)$ mmHg) and diastolic blood pressure ($80 (60 - 110)$ mmHg), serum creatinine (70.1 ± 12.2 μ mol/L), UAE ($9.8 (1.3 - 29.0)$ mg/24h) and eGFR (106.2 ± 15.1 ml/min/1.73m²) were within normal range for diabetic patients, whereas HbA1c (7.0 ± 1.4 %) and LDL cholesterol (2.8 ± 0.8 mmol/L) were slightly elevated. At baseline, 156 (70%) patients had no retinopathy and 67 (30%) had nonproliferative diabetic retinopathy (NPDR). After 48 months, 24 patients (10.7%) developed NPDR or progressed to proliferative diabetic retinopathy (PDR). From the 156 patients with no retinopathy at the beginning of the study 15 (9.6%) progressed to NPDR, while from the 67 patients with NPDR at the beginning of the study 9 (13.4%) progressed to PDR. Male sex (HR 2.9, CI 1.04-8.3, $p=0.04$), systolic blood pressure (HR 1.03, CI 1.01-1.06, $p=0.02$), UAE (HR 1.14, CI 1.06-1.23, $p=0.001$) and resting heart rate (HR 1.03, CI 1.01-1.07, $p=0.03$) were significantly associated with the development and progression of retinopathy.

Conclusion: The results of this study suggest that diabetic retinopathy may develop and progress in type 1 diabetic patients even without a coexisting renal disease. This points to the need for close monitoring of normoalbumi-

nuric type 1 diabetic patients aimed at early detecting, preventing or limiting the progression of retinopathy, especially in men with higher UAE, systolic blood pressure and higher resting heart rate.

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Incidence of severe diabetic retinopathy and all-cause mortality among migrants in Denmark

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Background and aims: Global migration has been increasing and it has been reported that migrants from non-European countries are usually diagnosed with diabetes more commonly and at younger age than European counterparts. Consequently, they may have higher rates of diabetic complications and death. Diabetic retinopathy (DR) is a common microvascular complication of diabetes and may progress to proliferative retinopathy and macular oedema if exposure of hyperglycemia and hypertension prolongs. Reports from UK and US showed that African and Asian migrants had higher prevalence of DR and especially sight-threatening retinopathy, than UK and US born people with diabetes. It has been considered that the difference probably reflects worse glucose and blood pressure control, lower frequency of eye examination and genetic background. However, longitudinal data on incidence of DR using nationwide data among migrants are lacking.

Materials and methods: Nationwide clinical data from the Danish Diabetes Database for Adults (Dansk Voksen Diabetes Database) on >17 years old diabetes patients followed since 2005 was linked through the personal ID number with data from the National Patient Register for identification of retinopathy diagnoses, the Cause of Death Register for information on deaths and the Central Personal Register for information on country of origin. We classified patients according to country of origin into six groups: Denmark, other Europe, Sub Saharan Africa, Middle East (includes North Africa), Asia and America (includes Oceania). From 73,572 patients with complete information, follow-up of 56,148 patients (mean age 58 ± 15 years, duration of diabetes 8.6 ± 9.3 years) and free from severe DR at baseline were analysed. We estimated event rates and hazard ratios (HR) for incidence of severe retinopathy and all-cause mortality between geographical regions.

Results: At baseline, median age, duration of diabetes and systolic blood pressure were lower and median HbA1c was higher in migrants from Sub Saharan Africa, Middle East and Asia compared to diabetes patients of Danish origin. During 215,565 person-year of follow-up, 6,348 patients suffered an incident of severe DR and 2,400 patients died. Compared to Danish borns, migrants from Middle East and Asia had higher risk of severe retinopathy (HR, 1.15 [95% confidence interval (CI) 1.04-1.27] and 1.24 [1.03-1.49], respectively) after adjustment for age, sex, type of clinic, body mass index, smoking status, and diabetes type, duration and control. The associations remained after further adjustment for blood pressure and lipids. Mortality rates were lower among those with other European, Sub Saharan, Middle Eastern, Asian or American origin compared to Danish borns after adjustment for age and sex (HR; 0.71, 0.26, 0.51, 0.55 and 0.52, all significant except America).

Conclusion: Migrants with diabetes from the Middle East and Asia showed higher risk of incidence of severe DR compared to Danish borns, but substantially lower mortality rates.

PS 102 Mechanisms of microangiopathy: experimental

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Topical administration of a GLP-1 agonist prevents retinal neurodegeneration in experimental diabetes

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Background and aims: Retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Therefore, treatments aimed at preventing or arresting retinal neurodegeneration has been recently proposed as a new strategy to reduce the incidence and progression of DR. There is evidence that glucagon-like peptide-1 (GLP-1) exerts a neuroprotective effect in the central nervous system but there is scarce information on this issue in the retina. The aim of the study was to explore the effectiveness of a GLP-1 analogue (liraglutide) administered topically (eye-drops) in preventing retinal neurodegeneration in db/db mice, an experimental model of type 2 diabetes.

Materials and methods: For this purpose we evaluated a total of 24 diabetic mice (db/db) aged 8 weeks that were randomly assigned to daily oral treatment with liraglutide (6 mg/ml) (n=12) or vehicle (n=12) for two weeks. Twelve non-diabetic mice (db/+) were used as control group. Retinal neurodegeneration was evaluated by measuring glial activation (immunofluorescence and Western blot) and apoptosis (TUNEL, cell count in ganglion cell layer). Functional abnormalities were assessed by electroretinography (ERG). **Results:** We observed that diabetic mice presented significantly higher glial activation and apoptosis than age-matched non-diabetic mice. The diabetic mice treated with eye drops of GLP-1 analogue presented a significant decrease of both glial activation and rate of apoptosis than diabetic mice treated with vehicle. Furthermore, a significant improvement of ERG parameters was observed.

Conclusion: Topical administration of a GLP-1 analogue prevents retinal neurodegeneration induced by diabetes. Our findings open up a new pharmacological strategy targeted to early stages of DR.

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Early-outgrowth bone marrow cells protect the retina of experimental model of type 2 diabetes by improving SIRT1 signalling

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Background and aims: Cellular therapy has been studied in cardiovascular disease but has rarely been investigated in eye diseases. Sirtuins (SIRT1), a family of deacetylases, is thought to be sensitive to oxidative stress. This study hypothesized that cell therapy using early outgrowth cells (EOC) could protect the diabetic retina through antioxidant means, thus improving the SIRT1 pathway. This study investigated the possible therapeutic effects of cells derived from healthy (db/m) and diabetic (db/db) animals on diabetic retinopathy (DR).

Materials and methods: Mice aged 8-weeks and db/db were randomized to receive a unique intravenous injection of PBS or 0.5 × 10⁵ db/m EOCs or 0.5 × 10⁵ db/db EOCs. Four weeks later, the animals were euthanized and the eyes enucleated. For in vitro study, an EOCs-conditioned medium (EOC-CM) was generated from db/m and db/db EOCs cultures. The rat Müller cells (rMCs) were exposed for 24 h to normal (NG), high glucose (HG) combined, or with neither db/m nor db/db EOC-CMs.

Results: In diabetic rats, there was an increase of DR and oxidative damage markers accompanied by an increase in NOX4 expression leading to detriment of SIRT1 protein. This was followed by lysine-310-p65-NFκB acetylation. The treatment with cells from db/m significantly reduced this damage, but the treatment with cells from db/db mice fully restored these alterations to normal levels. The rMCs exposed to HG displayed GFAP and VEGF expression upregulation accompanied by an increase in NOX4 expression, ROS levels and acetyl-lysine-310-p65-NFκB. Protein expression and activity of

SIRT1 were markedly reduced in diabetic milieu conditions. The treatment with both EOC-CMs prevented all these abnormalities, but db/db EOC-CM resulted in full restoration to NG conditions. The presence of EX-527, a SIRT1-specific blocker, or siRNA SIRT1 abolished the observed effects in both treatments.

Conclusion: This study demonstrates that the endocrine capacity of EOCs is effective in improving the retinal SIRT1 pathway, thus protecting the retina from diabetic milieu insult. This is one evidence that regenerative therapy for DR using EOCs or its released factors could be a promising tool for patients with diabetic eye disease.

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Attenuation of reactive gliosis in retinas by PARP-1 inhibitors treatment under diabetes in rats

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Background and aims: Diabetic retinopathy (DR) is a multifactorial disease, and persistent hyperglycemia appears to be a major contributor to its development. Gliosis is a hallmark of retinas neurodegeneration. The exact molecular mechanisms which contribute to development of diabetes-induced retinas neuropathy are not completely understood. The study was aimed to estimate the Poly(ADP-ribose)ylation and reactive gliosis under DR and effect of Poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors, 3-aminobenzamide (3-AB) and nicotinamide (NAM) on these processes.

Materials and methods: All studies were carried out after 10 weeks of diabetes (streptozotocin, 70 mg/kg of body weight, i. p.) in male Wistar rats treated 2 weeks with or without 3-AB (30 mg/kg/day, i. p.) and NAM (100 mg/kg/day, i. p.). The proteins content was assessed by Western blot and immunohistochemically.

Results: The levels of glial fibrillary acidic protein (GFAP) were significantly increased in retinas at diabetes as result of glial reactivity. In addition, expression of immunoreactive products of GFAP degradation (lower molecular weight polypeptides, in the range from 47 to 37 kDa), another representative indicator of reactive gliosis, was observed. Marked reduction of GFAP levels was seen in retinas of diabetic rats treated with 3-AB or NAM, though content of retinas GFAP did not reached to the control. Noteworthy, 3-AB and NAM administration completely prevented increase in GFAP degradation. Increased GFAP immunoreactivity was observed in retinas sections of diabetic rats compared with control, which is in parallel with data obtained by Western blot. Immunohistochemically, supplementation of diabetic rats with PARP inhibitors counteracted glial activation in retinas. Intensity of glial response appeared to be correlated directly with PARP-1 activation. Western blot analysis demonstrated significant upregulation of PARP-1 expression by about 1.5 in diabetic retinas compared to nondiabetic group. At the same time PARP-1 fragmentation was exacerbated more than 2-fold in diabetic rats as compared to control. PARP-1 inhibitors administration to diabetic rats normalized PARP-1 expression in retinas. However, effect of NAM was more profound than 3-AB not only on protein expression but also on protection of PARP-1 from its fragmentation due to its antioxidant property and improving of proinsulin biosynthesis through increase of NAD level that is responsible for the cellular ATP which is necerely for glycolysis and mitochondrial respiration. Poly(ADP-ribosyl)ated proteins (pADPs) were detected primarily in range from 72 to 130 kDa of the retinas protein spectrum, with several minor bands at 17 to 55 kDa. Both 72 to 130 kDa and 26 to 37 kDa pADPs expression was increased in diabetic rats as compared to control and was essentially prevented by 3-AB and NAM treatment.

Conclusion: Thus, PARP-1 inhibitors can be potential drugs for treatment of DR through improvement of PARP-1 fragmentation and modulation of reactive gliosis that maintain homeostasis and normal neuronal function in retinas under diabetes.

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p62 is not only a marker of autophagy but also a possible target against retinal Müller cells apoptosis under diabetic milieu conditions

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Background and aims: Inhibition of autophagy, a catabolic process by which cells degrade intracellular components inside lysosomes, induces degenerative changes in mammalian tissues that resemble those associated with aging, and was described to be altered in retinal cells under diabetic conditions. Apoptosis, or programmed cell death, appears to play an important role in the pathogenesis of diabetic complications. The aim of this study was to investigate the interaction between autophagy and apoptosis of retinal Müller cells under diabetic conditions *in vitro*.

Materials and methods: Rat Müller cells (rMCs) were cultured in normal glucose (NG; 5.5 mM), high glucose (HG; 30 mM) and under oxidative stress conditions (H_2O_2 ; 1 mM) for 24, 48, 72h, with and without N-Acetyl Cysteine (NAC), autophagosome formation inhibitor, 3-Methyladenine (3MA; 1mM) and PERK inhibitor I, GSK 2606414. The markers of autophagosome formation, Beclin1, and autophagosome-lysosome fusion, p62, were measured by Western blot (WB). Intracellular reactive oxygen species (ROS) were evaluated by 2'-7'-dichlorofluorescein (DCFH(2)) assay and apoptosis was assessed by TUNEL and extrinsic apoptotic pathway by caspase 8 activity.

Results: In rMCs, HG and H_2O_2 increased Beclin1 and p62 levels ($p<0.05$) as well as the rate of cellular apoptosis ($p<0.05$) in a time independent manner, and increased activity of caspase 8 ($p<0.05$). Whereas 3MA decreased the expression of Beclin1 under HG, it increased further p62 levels, caspase 8 activation and cellular apoptosis ($p<0.05$). The presence of GSK 2606414, an indirect inhibitor of phospho-eif2 α , a endoplasmic reticulum (ER) stress marker, decreased the activation of caspase 8 ($p<0.05$). NAC was able to prevent the increase of intracellular ROS ($p<0.05$) and reversed the increments of Beclin1 and p62 expressions, caspase 8 activity and cellular apoptosis ($p<0.05$).

Conclusion: Cells exposed to diabetic conditions displayed an alteration of autophagic flux as demonstrated by increased levels of Beclin1 and p62. The increases in p62 levels suggest a defective clearance of autophagosome by lysosome fusion. The accumulation of p62 levels cause activation of caspase 8 leading to retinal Müller apoptosis. The modulation of p62 levels could be a possible target to prevent retinal Müller cells apoptosis under diabetic conditions.

Supported by: Fapesp

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The association between Bsm1/Apa1 polymorphisms in the vitamin D receptor gene and complications of type 2 diabetes in the Korean population

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Background and aims: Type 2 diabetes is one of the most common disease in with devastating complications. However, genetic susceptibility of diabetic complications has not been clarified. The vitamin D endocrine system is related with calcification and lipolysis, insulin secretion, and may be associated with many complicated disease including diabetes and cardiovascular disease. Recent studies reported that single nucleotide polymorphisms (SNP) of vitamin D receptor (VDR) gene were associated with diabetic complications.

Materials and methods: We evaluated the association of VDR SNPs (BsmI and ApaI) with diabetic complications in 537 type 2 diabetic patients. PCR-restriction fragment length polymorphism was used to test the genotype and allele frequency of BsmI (rs1544410; BB, Bb, bb) and ApaI (rs7975232; AA, Aa, aa) polymorphisms.

Results: Patients with B allele (BB or Bb) was significantly associated with lower risk of severe retinopathy (severe nonproliferative diabetic retinopathy or proliferative retinopathy) (7.4%; 5/68) compared with patients without B allele (bb) (17.3%; 81/469) (Odds Ratio=2.63, $P=0.037$). This association was significant after adjusting for age, sex and BMI ($P=0.044$) in logistic regression analysis. In Kaplan-Meier survival analysis, there was a trend of lower risk of severe retinopathy among patients with B allele (BB or Bb) compared

with patients without B allele (bb) ($P=0.091$). Regarding coronary artery disease, patients with B allele (BB or Bb) had significantly higher risk of developing myocardial infarction compared with patients without B allele (bb) ($P=0.049$). No significant association was observed regarding ApaI SNP.

Conclusion: Our findings suggest that BsmI SNP in VDR gene is associated with lower risk of severe retinopathy and higher risk of coronary artery disease in type 2 diabetic patients. BsmI genotype could be used as a susceptibility marker to predict the risk of diabetes complications.

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Pro-angiogenic and anti-angiogenic microRNAs expression on endothelial progenitor cells from type 1 diabetic patients with and without diabetic retinopathy

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Background and aims: Endothelial progenitor cells (EPCs) are recognized as a key cell responsible for healthy maintenance of the vasculature, including the retina. Recently, microRNAs (miRs) have emerged as critical regulators of signalling pathways in multiple cell types including endothelial cells. miR-221 and miR-222 are highly expressed in vascular endothelial cells where they exert anti-proliferation, anti-migration and pro-apoptosis effects. miR-126 has been identified as pro-angiogenic and increases colony formation, proliferation, and migration of EPCs. The expression of miRs in EPCs from patients with type 1 diabetes mellitus (DM1) and its relation with different stages of diabetic retinopathy (DR) has not been reported to date. In addition, there is evidence that triglyceride lowering drugs such as fenofibrate can reduce progression of DR, but the mechanisms implicated remain unknown. Therefore, the aim of the present study was to analyze the expression of miR-222, miR-221, and miR-126 in circulating EPC from patients with DM1 with and without DR.

Materials and methods: Patients with DM1 without overt macrovascular disease, were recruited from January 2012 to December 2013. 33 DM1 patients with DR, 30 DM1 patients without DR, and 29 non-diabetic controls were included. Venous blood was collected for EPC colony culture and for flow cytometry analysis. Total RNA was extracted and purified and real-time qPCR was performed to analyse miRs expression after 7 days of EPC culture. Relative changes in miRs expression were analyzed with the 2⁻(delta delta C(T)) method. Non-parametric methods were used to compare differences between groups.

Results: There was no significant difference in age and gender distribution between patients and controls. Patients with DR were significantly older than those without DR (48.18 ± 10.33 vs 40.8 ± 11.104 , $p=0.009$) and had longer duration of diabetes ($34 [24-39]$ vs $17 [10.75-22.5]$ years, $p<0.0001$). Circulating EPC number was reduced in DM1 patients compared to controls ($p=0.006$) but was not significantly different in DM1 patients with and without DR. miR-126 and miR-221 expression was significantly higher in DM1 compared to controls ($p=0.024$ in both cases). In addition, miR-221 was significantly higher in patients with DR than in those without DR ($p=0.046$). However, differences between non proliferative and proliferative DR were not significant ($p=0.08$). We did not find any relationship between miRs expression and duration of diabetes, HbA1c, fasting glucose, albumin excretion ratio, or total, HDL and LDL cholesterol. There was a positive correlation between miR-126 and miR-221 expression and triglyceride concentration (Rho 0.266, $p=0.044$ and Rho 0.413, $p=0.01$, respectively). We found, also, a positive correlation between circulating EPC number and total and LDL cholesterol concentrations (Rho 0.306, $p=0.019$ and Rho 0.321, $p=0.013$, respectively).

Conclusion: Circulating EPC number is reduced in DM1 patients compared to controls. miR-221 expression is significantly higher in DM1 patients compared to controls and in patients with DR than in those without DR. In addition there is a positive correlation between miR-221 expression and triglyceride concentration.

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Thiamine metabolism abnormalities contribute to the progression of diabetic nephropathyK. Kankova¹, K. Kuricova¹, V. Dvorakova¹, L. Pácal¹, Z. Marcanova¹, J. Svojanovsky², J. Olsofsky², J. Belobradkova³, J. Rehorova³;¹Dept. of Pathophysiology, Masaryk University, ²Dept. of Internal Medicine, St. Anne University Hospital, ³Dept. of Gastroenterology, Faculty Hospital Brno-Bohunice, Brno, Czech Republic.

Background and aims: Pentose phosphate pathway (PPP) represents potentially protective pathway in hyperglycaemia since it can process glycolytic intermediates and thus decrease production of reactive dicarbonyls (methylglyoxal) and reactive oxygen species in mitochondria. Transketolase (TKT) is the rate-limiting enzyme of non-oxidative branch of PPP whose activity depends on thiamine diphosphate (TDP) - an active form of thiamine (vit. B1) - as a cofactor. Thiamine supplementation was shown to prevent development and progression of diabetic nephropathy (DN) in animal model of diabetes. Thiamine is delivered to the cell via specific thiamine transporters 1 (encoded by SLC19A2) and 2 (SLC19A3) and phosphorylated by thiamine pyrophosphokinase (TPK). We have previously shown that plasma levels of thiamine are dominantly influenced by GFR, however increasing plasma thiamine in subjects with decreased renal function are not paralleled by increase of intracellular TDP. The aim of the current study was to analyse relationship between plasma and erythrocyte parameters reflecting the thiamine status and progression of DN, cardiovascular morbidity and mortality. Additionally, since genetic variability in SLC19A2 and SLC19A3 loci may potentially affect activity of thiamine transport, common SNPs in those genes were detected and tested for their contribution to the progression of DN.

Materials and methods: Prospective observational cohort study comprised a total of 273 type 2 diabetics with variable stage of DN at baseline followed for a median of 39 [IQR 21 - 59] months. Following end-points were considered: (1) progression of DN by stage, (2) major cardiovascular event (MCVE, non-fatal and fatal myocardial infarction or stroke, limb amputation, revascularisation) and (3) all-cause mortality. Plasma and erythrocyte TDP was detected using HPLC. TKT activity was determined by kinetic method. Genotyping of 6 SNPs (3 in the SLC19A2 locus (rs1983546, rs7522245, rs6656822) and 3 in the SLC19A3 locus (rs13025803, rs4973216, rs7567984)) was performed by RT-PCR. Time-to-event analysis was carried out to ascertain contribution of thiamine, TDP and SNPs to studied end-points.

Results: Cumulative incidence of DN progression was 22.9%, CVE 8.2%, and ACM 19.8%. Significant differences in DN progression and all-cause mortality were ascertained for plasma TDP tertile groups (both $P < 0.001$) and for erythrocyte TKT activity for the latter end-point ($P = 0.01$). In all cases the highest tertiles were associated with the worst survival rate while the lowest tertile of erythrocyte TDP/plasma TDP was associated with the lowest survival rate ($P = 0.01$). No significant effects were ascertained for any of the studied SNPs and the three end-points (all $P > 0.05$).

Conclusion: Our results indicate that abnormalities of thiamine metabolism induced by diabetes, especially intracellular deficit of active TKT cofactor and impaired activity of TKT contribute to the progression of diabetes-associated morbidity or mortality. Elucidation of molecular mechanisms responsible for decreased intracellular availability of TDP is warranted.

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Defective mitochondrial turnover in experimental diabetic nephropathyG.C. Higgins^{1,2}, T.-V. Nguyen¹, S.A. Penfold¹, P.M. Robb¹, G. Ramm³, K.E. White⁴, R.W. Bilous⁴, M.E. Cooper^{1,5}, J.M. Forbes⁶, M.T. Coughlan^{1,5};¹Diabetic Complications, Baker IDI Heart and Diabetes Institute,²Department of Biochemistry & Molecular Biology, Monash University,³Biochemistry & Molecular Biology, Monash University, Clayton, Australia,⁴EM Research Services, Faculty of Medical Sciences, Newcastle University,Newcastle, UK, ⁵Department of Medicine, Monash University, Melbourne,⁶Glycation & Diabetes, Mater Medical Research Institute, South Brisbane, Australia.

Background and aims: Diabetic nephropathy (DN) is the major cause of end stage renal disease in the Western world. Defects in mitochondrial bioenergetics are evident in DN and are thought to initiate renal impairment. Accumulation of fragmented mitochondria are found in the renal cortex in experimental diabetes, suggesting that in tandem with a shift in dynamics, mitochondrial clearance mechanisms may be impaired. The process of mi-

tophagy is the selective targeting of damaged or dysfunctional mitochondria to autophagosomes for degradation through the autophagy pathway. Here, we aimed to determine if there was an impairment in mitophagy and changes in mitochondrial dynamics in the kidney in DN.

Materials and methods: Markers of mitophagy and mitochondrial dynamics were followed in the renal cortex from mice rendered diabetic with the beta cell toxin streptozotocin (STZ). Tissue from the renal cortex was studied by western immunoblot for changes in mitophagy and mitochondrial dynamics changes, while mitochondrial bioenergetics were performed to measure mitochondrial function. Electron microscopy was also undertaken to follow changes in mitochondrial morphology, between control and diabetic populations. Human renal biopsies, taken from both type 1 and control patients (taken from kidney donor patients) were studied by electron microscopy for evidence of changes in mitochondrial morphology and autophagy/mitophagy.

Results: Bioenergetics were impaired in mitochondria isolated from renal cortex, with a decline in ATP content, increased reactive oxygen species and depolarisation of the inner mitochondrial membrane. Increased recruitment of Drp-1 to mitochondria was observed in diabetes, with a 3-fold increase in Drp-1 translocating to mitochondria ($n = 6$, $P < 0.05$), indicating a shift towards mitochondrial fission. Electron microscopy imaging revealed mitochondrial fragmentation in the proximal tubule epithelial cells (PTECs). Mitophagy impairment was seen with a decrease in autophagic flux (decline in LC3-II), with a 2-fold decrease ($n = 6$, $P < 0.05$) in renal cortical cell lysates, coupled with a decline in Parkin translocation to mitochondria (approximate 2-fold decrease, $n = 6$, $P < 0.05$) and an approximate 10-fold decrease of p62 within mitochondrial fractions ($n = 6$, $P < 0.05$). Importantly, these data correlate with findings from renal biopsies of patients with DN that show striking changes in morphology of mitochondria residing within PTECs manifesting as a greater percentage of fragmented mitochondria (3-fold increase, $n = 6$, $P < 0.0001$), indicative of a shift towards fission.

Conclusion: These data demonstrate that in chronic hyperglycaemia, mitochondria undergo fission, however, there is a defect in mitophagy, leading to reduced mitochondrial turnover and accumulation of dysfunctional mitochondria. A better understanding of the cellular and molecular events that govern mitophagy and dynamics in DN may lead to improved therapeutic strategies.

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Nrf2 overexpression inhibits mechanical stretch-induced fibronectin expression

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Background and aims: Mesangial cells are a primary target for haemodynamic perturbations in hypertensive glomerulopathies (e.g. diabetes). Oxidative stress plays a central role in the pathogenesis of diabetic microvascular complications, and the transcription factor Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) has been proposed as a central player in the cellular antioxidant response. The aim of this study was to evaluate, in cultured human mesangial cells (HMCs), the role of Nrf2 in the stretch-mediated antioxidant response and fibronectin secretion.

Materials and methods: HMCs were exposed to 10% average elongation (S) or static conditions (NS) up to 48h. Cellular superoxide was assessed with Cytochrome-C reduction assay, while mitochondrial superoxide was investigated using MitoSOX Red. Protein and mRNA expression were assessed respectively with western immunoblotting and real time PCR after normalisation against housekeeping genes. Fibronectin secretion was assessed by ELISA and normalised against cell number.

Results: S increased superoxide production ($p=0.01$), which was paralleled by an upregulation of the antioxidant enzyme heme oxygenase-1 (HO-1) both at the mRNA and protein level ($p=0.01$). The increase in superoxide was not accompanied by an increase in p22phox, p67phox or Nox4 mRNA expression, whilst mRNA expression of gamma-glutamyl cysteine ligase catalytic subunit was upregulated ($p=0.05$), but not mRNA levels for NAD(P)H quinone oxidoreductase-1 or glutathione peroxidase. Stretch-mediated increase in superoxide production was significantly inhibited by the NADPH-oxidase inhibitors apocynin (10 mM) and diphenyleneiodonium (100 mM), and by the antioxidant rotenone (25 nM), suggesting a mitochondrial origin of oxidative stress in stretched cells. Inhibition of stretch-mediated superoxide production was paralleled by absent stretch-induced HO-1 protein upregulation. Transfection of HMCs with dominant negative Nrf2 resulted in inhibition of stretch-induced HO-1 upregulation ($p=0.05$), but had no effect on basal and stretch-induced (24 and 48h) fibronectin secretion when compared to control (GFP-transfected) cells. Conversely overexpression of wild type Nrf2 resulted in a significant HO-1 up regulation ($p=0.01$), which was paralleled by a reduction in both basal and stretch-induced fibronectin ($p<0.01$).

Conclusion: Mechanical stretch-mediated superoxide production in HMCs is paralleled by an upregulation of HO-1. Nrf2 plays a role in stretch-mediated HO-1 upregulation. Overexpression of Nrf2 (and parallel HO-1 upregulation) is sufficient to prevent basal and stretch-induced fibronectin secretion in HMCs in vitro.

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Development of impaired renal function in pre-diabetic mice overexpressing SREBP-1c

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Background and aims: Microalbuminuria associates with insulin resistance even in humans without overt type 2 diabetes. However, development of albuminuria and altered renal function remains inadequately understood in the context of insulin resistance. Here, we examined a specific model of insulin resistance with partial lipodystrophy and ectopic lipid deposition. Transgenic aP2-SREBP-1c mice overexpress sterol regulatory element binding protein 1c (SREBP-1c) in adipose tissue, and are hyperinsulinemic and insulin resistant based on hyperinsulinemic-euglycemic clamps, when compared to C57BL/6 mice serving as controls.

Materials and methods: Urinary albumin-to-creatinine ratio was measured by ELISA. Kidneys from sacrificed mice were weighed and homogenized for assessing lipid peroxidation by thiobarbituric acid (TBARS).

Results: The aP2-SREBP-1c mice had 2.5-fold higher urinary albumin-to-creatinine ratio than control mice (15.8 ± 4.5 μg albumin/ μg creatinine vs. 6.2 ± 0.8 μg albumin/ μg creatinine, $P<0.01$, $n=6$). The transgenic mice showed higher kidney weight as compared to controls (394 ± 13 mg vs. 299.9 ± 13 mg, $P<0.01$, $n=6$) indicating renal hypertrophy. Similarly, kidney-to-body weight ratio was also higher in the transgenic mice ($1.22\pm 0.01\%$ vs. $1.12\pm 0.03\%$, $P<0.01$, $n=6$). In kidney homogenates of transgenic mice, TBARS were almost twice compared to controls (0.61 ± 0.08 $\mu\text{mol}/\text{mg}$ protein vs. 0.32 ± 0.02 $\mu\text{mol}/\text{mg}$ protein, $P<0.05$, $n=6$).

Conclusion: In conclusion, insulin resistant but nondiabetic aP2-SREBP-1c transgenic mice display impaired renal function and increased lipid peroxidation suggesting an oxidative stress response. The aP2-SREBP-1c transgenic mice may therefore serve as a novel rodent model of pre-diabetic kidney disease.

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Inactivation of Wnt pathway and interaction of GSK3 β with p53 contribute to podocyte apoptosis in diabetes mellitus and are prevented by treatment with green tea

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Background and aims: In diabetes mellitus (DM) podocyte apoptosis contributes to albuminuria and renal disease progression. Green tea (GT) reduced albuminuria in diabetic rats by an unknown mechanism. The mechanism of podocyte apoptosis in DM is not well understood. Wnt pathway is a key signaling in integrating cell adhesion, motility, cell death and differentiation in podocytes, and GSK3 β plays a crucial role in this pathway. Activated GSK3 β is known to promote p53-mediated increases in apoptotic signaling, by functional interaction GSK3 β -p53 after camptothycin-DNA damage, in human neuroblastoma and lung carcinoma cells. The contribution of the interaction GSK3 β -p53 leading to podocyte apoptosis under DM is not known. Aims of the present study were to assess the contribution of Wnt pathway, principally by the interaction of GSK3 β with p53 in podocyte apoptosis and the effects of GT in these abnormalities.

Materials and methods: For in vitro studies, immortalized mouse podocytes (iMPs) were cultured in normal glucose (NG; 5.5 mM) and high glucose (HG; 30 mM) with or without GSK3 β blocker (BIO), p-LRP6 blocker (DKK-1), siRNA for LRP6. We also tested if GT activates Wnt. Apoptosis was assessed by caspase-3 activity and TUNEL. GSK3 β -p53 interaction, and GSK3 β and p-53 relations with Wnt components were done by immunoprecipitation (IP). In vitro filtration barrier integrity was done by albumin influx assay. For in vivo studies, diabetic 12 week-old spontaneously hypertensive rats (SHR) received or not daily GT treatment (1.7g/Kg body weight/day). After 12 weeks of treatment, albuminuria was quantified by ELISA kit. Nephron, Wnt pathway components (p-LRP6 and GSK3 β (p-Ser9) and p-p53 levels were measured by immunofluorescence and Western blot (WB). Podocyte foot processes were assessed by electron microscopy.

Results: In iMPs, HG inactivated Wnt by reducing p-LRP6 and WNT-1 component expression ($p<0.0001$). Consequently, GSK3 β was activated ($p=0.003$) and GSK3 β -p53 interaction led to rise in apoptosis and albumin permeability flux, all of which were ameliorated by GT. NG treatments with Wnt blockers, such as: BIO, DKK-1 and siRNA LRP-6 had the same effects as HG. In the rats, systolic blood pressure did not differ among the groups. Body weight was less and glycemia was greater in diabetic rats (treated or not with GT). In diabetic animals, rise in albuminuria ($p=0.0083$) was in parallel with reduction of nephrin ($p=0.0002$) and synaptopodin ($p=0.038$), and increased in podocyte foot process effacement and in p-p53 level ($p=0.011$). All these parameters were prevented by GT ($p<0.0001$; $p=0.0016$; $p=0.003$, respectively). p-LRP6 and pGSK3 β levels were reduced in diabetic rats ($p=0.044$ and $p=0.0002$, respectively) and they were all reversed by GT ($p=0.05$ and $p=0.006$, respectively). IPs showed a decrease in synaptopodin-LRP6 ($p=0.048$) and synaptopodin-GSK3 β ($p=0.0008$) interactions in diabetic animals, suggesting blockade of podocyte Wnt pathway, in vivo. GT reestablished these interactions. ($p=0.0004$ and $p<0.0001$, respectively).

Conclusion: In summary, podocyte Wnt blockade leads to high GSK3 β -p53 interaction promoting podocyte apoptosis under diabetic conditions. Activation of Wnt pathway and inactivation of GSK3 β -p53 may be important strategies to prevent podocyte apoptosis in diabetes mellitus.

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Global toll-like receptor 4 knockout attenuates features of diabetic nephropathyI. Jialal¹, S. Devaraj²;¹Internal Medicine, UC Davis Medical Center, Sacramento,²Pathology, Baylor College of Medicine, Houston, USA.

Background and aims: Type 1 Diabetes Mellitus (T1DM) is a pro-inflammatory state with increased Toll-like receptor (TLR) activity. Inflammation is crucial in diabetic vascular complications including diabetic nephropathy (DN). We tested the effect of a global deficiency of TLR4 on renal inflammation, fibrosis and podocytopeny.

Materials and methods: Streptozotocin (STZ) was used to induce diabetes in wildtype (WT) and TLR4-knockout (TLR4KO) mice. Control (C) and diabetic groups (STZ-WT and STZ-TLR4KO) mice were euthanized at 17 weeks and plasma and kidneys collected.

Results: Compared to C, the STZ-WT group had significantly increased macrophage and TLR4 immunostaining in the kidney, significant increases in MyD88, Interferon Regulatory Factor 3, Nuclear Factor Kappa B activity, Tumor Necrosis Factor Alpha, Interleukin 6, and MCP-1 protein levels; all these parameters were significantly decreased in the STZ-TLR4KO compared to STZ-WT mice. Compared to C, there were significant increases in fibrosis markers including Collagen 4, Laminin and Transforming Growth Factor Beta in STZ-WT which were significantly decreased in the STZ-TLR4KO versus the STZ-WT. Podocyte numbers and podocin protein were decreased in the STZ-WT versus C and these were increased in the STZ-TLR4KO mice.

Conclusion: In conclusion, a global genetic deficiency of TLR4 also ameliorates renal inflammation, fibrosis and podocytopeny and could be important in DN.

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Alkalinization attenuates diabetes-induced albuminuria and glomerular injury through reduced oxidative stressT. Senmaru¹, M. Fukui¹, M. Tanaka¹, H. Okada^{2,1}, T. Fukuda¹, K. Mitsuhashi¹, N. Kitagawa¹, T. Kimura¹, Y. Hashimoto¹, N. Nakanishi¹, M. Yamazaki¹, G. Hasegawa^{2,1}, N. Nakamura¹;¹Department of Endocrinology and Metabolism, Kyoto Prefectural University of Medicine Graduate School of Medical Science,²Division of Metabolism, Nephrology and Rheumatology, Japanese Red Cross Kyoto Daini Hospital, Japan.

Background and aims: Patients with diabetes frequently have chronic metabolic acidosis. Metabolic acidosis traditionally defined as a decrease in blood bicarbonate (HCO₃⁻) concentration contributes to the progression of diabetic nephropathy, leading to end-stage renal failure. However, it is not well known whether alkalinization could prevent the progression of diabetic nephropathy, and if so, its mechanism has not been elucidated. In this study, we investigated the effects of alkalinization by potassium-sodium citrate on the pathogenesis and progression of diabetic nephropathy.

Materials and methods: Diabetes was induced using streptozotocin in male C57BL/6 (WT) mice at 7 weeks of age. Diabetic mice were divided into two groups: alkalinization (Alkali+) and non-alkalinization (Alkali-). Alkali+ was allowed free access to water containing potassium-sodium citrate (0.2% potassium citrate and sodium citrate hydrate) for 16 weeks.

Results: Blood HCO₃⁻ concentration is higher in Alkali+ than in Alkali- (25.1 ± 1.8 vs. 19.5 ± 1.5 mmol/L, *p* < 0.05). Urinary albumin excretion was decreased in Alkali+ compared to Alkali- (45.6 ± 7.1 vs. 99.8 ± 14.1 µg/day, *p* < 0.05). Diabetic mice exhibited glomerular hypertrophy and mesangial expansion as compared with WT mice. Histological and morphometric analysis of periodic acid-Schiff stained sections revealed that an increase of mesangial area was milder in Alkali+ than in Alkali- (26.2 ± 1.1 vs. 36.5 ± 1.0 %, *p* < 0.0001). In addition, the number of podocytes and the number of apoptosis cells was decreased in Alkali+ compared to Alkali-. On the other hand, alkalinization did not affect tubulointerstitial fibrosis (Sirius red stain). The degree of oxidative stress assessed by 4-hydroxynonenal (4HNE) immunostaining in the glomerular area was decreased in Alkali+ compared to Alkali-. Similar to the degree of 4HNE expression, the mRNA expression of p67-phox was decreased in Alkali+ compared to Alkali-. Furthermore, the mRNA expression of TGF-β and vascular endothelial growth factor was down-regulated in Alkali+. It is conceivable that the decreased expression of

these fibrogenic cytokine contributes to the prevention of advanced glomerular injury in Alkali+.

Conclusion: These results indicate that alkalinization attenuates diabetes-induced albuminuria and glomerular injury. Reduction of oxidative stress may be mainly involved in these changes. Alkalinization could be a useful treatment for the progression of diabetic nephropathy.

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Zinc exerts anti-fibrotic action by inhibiting TGF-beta1-induced type I collagen and fibronectin in human renal proximal tubular cells

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Background and aims: Hyperglycemia, chronic hypoxia and TGF-β1 contribute to the development of diabetic nephropathy. The aim of this study is to investigate whether novel key factors contribute to the pathophysiological mechanism of diabetic nephropathy.

Materials and methods: We analyzed microarray data using Affymetrix GeneChip (Rat Gene 1.0 ST Array) with microdissected juxtamedullary proximal tubules in a rat model of type 2 diabetes, Zucker Diabetic Fatty (ZDF) rats. Then, we studied the regulation of the expression of the candidate using human renal proximal tubular epithelial cells (HRPTECs).

Results: A total of 27,342 transcripts were analyzed, among them, 47 were upregulated (>2.0-fold) in the ZDF rat compared with the lean control rat. One of them which were upregulated in diabetic renal tubules was metallothionein (MT)-1. MT is a cysteine-rich protein with low molecular weight, and act as an antioxidant against the toxicity of metals, ischemia, and ROS. MT originally has a pivotal role in the regulation of the metabolism of zinc. Previous studies reported that diabetic patients showed decreased plasma level of zinc, and zinc might be involved in diabetic nephropathy. Therefore, to explore the interaction of MT and zinc in diabetic nephropathy, we next studied the regulation of MT expression, and the effects of zinc on MT expression. High glucose (25.5 mM) failed to affect MT-1 mRNA expression in HRPTECs, and insulin (100 nM) significantly enhanced MT-1 mRNA levels. Hypoxia (1% O₂) slightly increased MT-1 mRNA levels. 2.5 ng/ml TGF-β1 significantly decreased MT-1 mRNA levels compared to that of the control (0.62 ± 0.16 vs. control, *p* < 0.05). This inhibitory effect of TGF-β1 was not affected by the pretreatment of the inhibitors for ERK (PD98059) and JNK (SP600125) pathways, respectively. In contrast, the p38MAPK inhibitor (SB203580) apparently diminished the inhibitory effect of TGF-β1 on MT-1 mRNA. Unexpectedly, TGF-β1 increased another isoform of MT, MT-2 mRNA (1.86 ± 0.49 vs. control, *p* < 0.05), and the stimulatory effects of TGF-β1 on MT2 was inhibited by ERK inhibitor. On the other hand, zinc at 100 µM concentration remarkably increased MT-1 mRNA (17.3 ± 7.83 vs. control, *p* < 0.05), and restored the inhibitory effect of TGF-β1 on MT-1 mRNA in a dose-dependent manner (0–100 µM). Similarly, zinc augmented MT-2 expression in a dose-dependent manner, and 100 µM zinc significantly increased MT-2 mRNA (7.00 ± 2.76 vs. control, *p* < 0.05). TGF-β1 is known to be a profibrotic molecule which promotes renal fibrosis in diabetic nephropathy. Accordingly, 2.5 ng/ml TGF-β1 significantly increased mRNA levels of type I collagen (7.07 ± 1.48 vs. control, *p* < 0.05) and fibronectin (3.00 ± 0.88 vs. control, *p* < 0.05) in HRPTECs. Intriguingly, 75 µM zinc significantly suppressed TGF-β1-induced type I collagen mRNA down to 61% (*p* < 0.05), and 50 µM zinc also decreased TGF-β1-induced fibronectin-1 mRNA down to 65% (*p* < 0.05) of those in TGF-β1 alone treatment, respectively.

Conclusion: In conclusion, the current study showed that MT may be dysregulated in diabetic nephropathy, and suggesting that zinc might have the potential therapeutic effects in diabetic nephropathy by anti-TGF-β1 action and via the induction of antioxidant MTs.

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Protective effects of adiponectin on uncoupling of glomerular VEGF-NO axis in type 2 diabetic ratsN. Huang¹, F. Han², J. Zhao¹, N. Hou¹, X. Liu¹, X. Sun¹;¹Department of Endocrinology, ²Department of Pathology, Affiliated Hospital of Weifang Medical University, China.

Background and aims: The uncoupling of glomerular vascular endothelial growth factor (VEGF) - nitric oxide (NO) axis is an important mechanism of early diabetic nephropathy. We aimed to determine whether adiponectin

could reduce microalbuminuria and provide renal protective effects by improving endothelial dysfunction and uncoupling of glomerular VEGF-NO axis in streptozotocin-induced type 2 diabetic rats.

Materials and methods: Wistar rats were randomly divided into a normal control (NC) group, a diabetic nephropathy (DN) group induced by high-fat feeding and streptozotocin, diabetic rats injected with adenovirus-expressed adiponectin AD-AdipoQ-IRES-EGFP (AD-AdipoQ, DA group), and diabetic rats injected with AD-IRES-EGFP (AD-IRES, DI group). Blood and urine samples were collected. Endothelium-dependent vasodilatation (EDV) of the aorta was measured. Renal tissues were collected for CD34 immunohistochemistry. Glomerular NO and VEGF levels were measured by the Griess reaction and western blot testing, respectively.

Results: STZ-injected rats in the DN group had severe hyperglycemia and high levels of insulin ($P < 0.01$). This was reduced by intravenous injections of AD-AdipoQ (glucose, 11.3 ± 5.5 mmol/L vs 21.2 ± 3.3 mmol/L; insulin, 16.70 ± 2.40 uU/ml vs 20.91 ± 2.78 uU/ml, $P < 0.01$). Serum hs-CRP and MDA levels increased and serum adiponectin levels decreased significantly in the DN group compared with the NC group ($P < 0.01$). Intravenous injections of AD-AdipoQ reduced serum hs-CRP and MDA levels and increased serum adiponectin levels in diabetic rats (hs-CRP, 1.85 ± 0.56 mg/L vs 3.13 ± 0.38 mg/L; MDA 2.44 ± 0.28 umol/L vs 4.80 ± 0.59 umol/L; adiponectin, 273.50 ± 21.20 ug/L vs 94.97 ± 25.12 ug/L, $P < 0.01$). Albumin-to-creatinine (ACR) was significantly higher in rats in the DN group than those of the NC group [180.35 (113.54 – 233.29) mg/g vs 13.15 (8.93 – 17.95) mg/g, $P < 0.01$]. Injections of AD-AdipoQ significantly reduced the ACR in diabetic rats. [72.0 (46.18 – 99.41) mg/g vs 180.35 (113.54 – 233.29) mg/g, $P < 0.01$], indicating an improvement of early diabetic nephropathy. Severe EDV impairment was observed in the DN group, which was improved by AD-AdipoQ. CD34 expression in the glomeruli was also enhanced in diabetic rats, indicating increased proliferation of glomerular endothelial cells [mean optical density (IOD/area): 0.145 ± 0.015 vs 0.073 ± 0.007 , $P < 0.05$]. However, treatment with AD-AdipoQ partly improved the increased proliferation of endothelial cells in glomeruli (IOD/area: 0.113 ± 0.012 vs 0.145 ± 0.015 , $P < 0.05$). Diabetic rats showed increased glomerular VEGF levels and reduced NO levels ($P < 0.05$). This uncoupling of the VEGF-NO axis was partially improved by AD-AdipoQ ($P < 0.05$). There were no significant differences in these parameters between the DN and DI groups ($P > 0.05$).

Conclusion: Adiponectin reduces the degree of microalbuminuria and has renal-protective effects by improving endothelial dysfunction and uncoupling the glomerular VEGF-NO axis in early diabetic nephropathy.

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Heat shock proteins role in the biochemical and molecular interplay leading to diabetic nephropathy

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Background and aims: Heat shock protein (Hsp) expression is correlated with oxidative stress, yet few studies focus on Hsp's modulation in diabetes. The objective of this study was to highlight the link between Hsp levels and the biochemical changes leading to diabetic nephropathy, using the human embryonic kidney cells HEK293.

Materials and methods: Cells were treated for 12, 24 and 48 h with 200 µg/ml glycated-bovine serum albumin (AGE-BSA) or BSA. Reduced glutathione (GSH), advanced glycation lipid products (ALEs) levels and enzyme activities were evaluated by spectrophotometric methods. Superoxide dismutase (SOD) was also assessed by zymography. The receptor for advanced glycation end products (RAGE), Hsp 27, 60 and 70 expressions were evaluated by western immunoblotting and real-time PCR.

Results: AGE-BSA induced a time dependent RAGE upregulation. Maximal increases of gene expression by 2.25 fold and protein expression by 2.01 fold were registered after 48 h. Increased expression of RAGE has been documented in chronic renal disease and diabetic complications. Probably the up-regulated RAGE stimulated pro-inflammatory signaling and reactive oxygen species (ROS) production. Increased ROS contribute to GSH depletion by 70 % after 48 h, which hinders the cellular antioxidative and anti-AGE activities. SOD activity upregulated with AGE-BSA exposure time and increased by 33 % after 48 h. Zymogram analysis revealed a similar pattern. Cu/Zn-SOD had the most important contribution for the SOD activity after 12 h of exposure.

Probably the initial superoxide anion source is not mitochondrial but cytosolic, although later, Mn-SOD increased, indicating mitochondrial damage. After 24 h and 48 h, ALEs arose by 1.75 and 2.29 fold, suggesting oxidative damage is amassed with time. Catalase activity increased over 200 % after 12 h of AGE exposure and remained over 50 % higher than controls later on. This early increase denotes the presence of high hydrogen peroxide amounts, probably generated by NADPH oxidase rather than SOD activity. AGE exposure boosted glutathione peroxidase activity by 50 % after 48 h. This enzyme could be involved in the detoxification of lipid hydroperoxides that are most abundant after 48 h. Glutathione reductase and glucose 6-phosphate dehydrogenase upregulated by 35 % after 48 h. These enzymes are sensitive to ROS, and probably their increase was supported by Hsp70 activity. Hsp 70 gene and protein expressions augmented in a time dependent manner, increasing by 2.07 and 2.78 fold after 48 h. Hsp 70 expression seems a reliable indicator of the biochemical alterations described mainly by the increase of RAGE, ROS and ALE. Hsp 27 gene and protein expressions increased after 12 h of treatment by 1.93 and 1.52 fold, but decrease to 0.45 and respectively 0.16 fold after 48 h. Hsp 60 analysis revealed a similar profile. Hsp 27 and 60 decrease after 48 h suggests the possibility of pro-apoptotic pathways activation. **Conclusion:** ROS induced by AGE - RAGE activation employ antioxidative mechanisms, which are themselves susceptible to oxidative damage. Although Hsps protective roles can improve some of the AGE-induced effects, therapeutic approaches based on Hsp overexpression must take great care not to target anti-apoptotic Hsps, because inhibiting AGE-promoted apoptosis could invoke cancerous transformation.

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Lentiviral vector-mediated FoxO1 overexpression inhibits extracellular matrix protein secretion under high glucose conditions in mesangial cells

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Background and aims: The excessive accumulation of ECM proteins in the mesangium of glomerulus results in glomerulosclerosis and contributes to the genesis and development of diabetic nephropathy (DN). The bioactivity of Forkhead transcription factor O1 (FoxO1) is attenuated in renal cortex under high glucose conditions, which may be associated with the progression of DN. High glucose conditions also upregulate the expression level of ECM proteins by activating the transforming growth factor β (TGF- β)/Smad pathway in MCs. The study is designed to investigate whether FoxO1 is involved in the redundant secretion of extracellular matrix (ECM) proteins in mesangial cells (MCs) under high glucose conditions.

Materials and methods: Constitutively active FoxO1 recombinant lentiviral vectors (LV-CA-FoxO1) and GFP lentiviral vectors (LV-NC-GFP) were constructed. MCs were transfected with either LV-CA-FoxO1 or LV-NC-GFP cultured in high glucose medium (25 mmol/L), compared with untransfected cells cultured in high or normal glucose medium (5.6 mmol/L). Functional analysis was performed by real-time PCR, western blotting, immunofluorescence and flow cytometry to evaluate the expressions of FoxO1, ECM components and TGF- β signaling pathways.

Results: High glucose conditions increased p-FoxO1 protein levels and the ratio of p-FoxO1/FoxO1, thereby attenuating bioactivity of endogenous FoxO1, which was accompanied by marked increases in the expression of ECM components, including FN and Col I. High glucose conditions also activated TGF- β -mediated pathways by elevating the latent and mature protein levels of TGF- β 1, in accordance with the increase of TGF- β 1 mRNA levels. The expressions of TGF- β RI and TGF- β RII as well as the expression and phosphorylation of Smad3 were both increased under high glucose conditions, thereby promoting signal transduction of TGF- β -mediated pathways. Immunofluorescence analysis indicated that high glucose conditions increased p-Smad3 staining in the nucleus, consistent with an overall increase in p-Smad3 expression. By contrast, FoxO1 expression markedly increased and the ratio of p-FoxO1/FoxO1 decreased after LV-CA-FoxO1 transfection, accompanied by reductions of FN and Col I mRNA levels. The mRNA level of TGF- β 1 as well as its latent and mature protein levels both reduced with overexpression of transfected CA-FoxO1, while TGF- β RI and TGF- β RII levels decreased simultaneously. Although mRNA and protein levels of Smad3 remained unchanged after transfection, the phosphorylation of p-Smad3 was blocked and the ratio of p-Smad3/Smad3 declined to the level of which under normal glucose conditions. In addition, overexpression of CA-FoxO1 attenu-

ated nuclear staining of p-Smad3, in concert with faintly observed immunofluorescence in the cytoplasm, indicating lower basal expression of p-Smad levels and diminished transcriptional activity.

Conclusion: High glucose conditions increased the expression of fibronectin and collagen I by activating TGF- β /Smad3 pathway in mesangial cells, while lentiviral vector-mediated overexpression of constitutively active FoxO1 blocked this effect by suppressing the activation and signaling of the TGF- β /Smad3 pathway. FoxO1 may play a vital role in attenuating ECM protein secretion and alleviating the pathological changes associated with diabetic nephropathy.

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The experimental study of the effects of FoxO1 on the podocyte injury in diabetic rats

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Background and aims: Diabetic nephropathy (DN) is the chief reason for end-stage renal disease (ESRD). The podocyte injury of DN is often characterized by foot process fusion and effacement, podocytes getting smaller, negative charge becoming less, and podocytes detaching from glomerular basement membrane (GBM) and discharging in the urine. Nowadays, the importance of podocyte injury in pathogenesis of DN and the occurrence of proteinuria and glomerular sclerosis has been gradually acknowledged. We have previously demonstrated that the activity of forkhead transcription factor O1 (FoxO1) was decreased in the renal cortex of DN rats, and discovered that compared with DN rats without treatment, the expression of FoxO1 phosphorylation was reduced, the activity of FoxO1 was increased and the podocyte injury was improved in the renal cortex of DN rats with treatment. So it is inferred that the activity of FoxO1 may affect the podocyte injury in DN rats. Our study was performed to investigate the effects of FoxO1 on the podocyte injury in diabetic rats.

Materials and methods: Streptozotocin-induced diabetic rats were divided into three groups: diabetic rats (group d), rats transfected with empty lentiviral vectors (DM plus LV-empty-FoxO1 group, served as group a) and rats which were transfected with constitutively active FoxO1 recombinant lentiviral vectors (DM plus LV-CA-FoxO1 group, served as group b). The rats which received an injection of diluent buffer served as normal control (group c). 2,4,8 weeks after the infection, the levels of urine albumin, blood glucose, blood urea nitrogen and serum creatinine were measured. Real-time PCR and Western blotting were performed to measure the mRNA and protein expressions of FoxO1, podocalyxin, nephrin, desmin, COL4A3, COL4A5 and FoxO1 phosphorylation (p-FoxO1) level of renal cortex in rats. Moreover, light microscope and electron microscope were used to observe the structure changes of glomerulus and podocytes.

Results: Compared with group a and d, in group b, there was a significant increase in the mRNA and protein expressions of FoxO1, and a distinctly decrease in the levels of urine albumin, blood urea nitrogen and serum creatinine of rats (except ones in two weeks) (all $p < 0.05$, F values at the end of 8 weeks were 44001.312, 1557.130, 126.153, 498.800, 186.960, respectively), the mRNA and protein expressions of podocalyxin and nephrin all increased (all $p < 0.05$, F values of mRNA at the end of 8 weeks were 199.813, 116.899, respectively; F values of protein at the end of 8 weeks were 344.917, 353.486, respectively), the mRNA and protein expressions of COL4A3, COL4A5 and desmin all decreased (all $p < 0.05$, F values of mRNA at the end of 8 weeks were 665.64, 784.015, 579.257, respectively; F values of protein at the end of 8 weeks were 91.121, 122.954, 266.640, respectively), and pathological changes in kidney were also improved.

Conclusion: Upregulate the expression of FoxO1 by transfecting with constructed lentiviral vectors can definitely improve the podocyte injury in diabetic rats.

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Meta-analysis of genome-wide gene expression data from glomeruli in human kidney with diabetic nephropathy

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Background and aims: Genetic association studies have successfully revealed a number of important genes associated with diabetic nephropathy (DN). However, these DN-associated genes do not fully elucidate the mechanisms underlying the progression of the disease. In this report, we attempted to provide an additional catalog of functionally important candidate genes for DN by meta-analyzing genome-wide gene expression data from human glomeruli.

Materials and methods: We performed a gene-expression based genome-wide association study (eGWAS; searching for genes repeatedly implicated in functional microarrays) using 67 DN case-control microarrays from glomeruli in human kidney biopsy samples.

Results: We identified 429 significantly up-regulated genes and 323 down-regulated genes in DN glomeruli (Fig.1; $P < 2.2 \times 10^{-6}$). Pathway analysis highlighted the extracellular matrix (ECM) and receptor interaction, natural killer cell mediated cytotoxicity, and toll-like receptor signaling pathway (TLR1, TLR2 and MYD88) in up-regulated genes. Gene Ontology (GO) term analysis revealed the enrichment of "regulation of epithelial cell proliferation" in down-regulated genes. We also found ERBB4 gene was significantly down-regulated in patients with DN, together with the other related genes in ErbB signalling pathway, such as CDKN1B, SOS2, PLCG2 and CAMK2G.

Conclusion: Our studies have cataloged multiple gene-expression signatures that may play a role in the pathogenesis of DN or could serve as biomarkers.

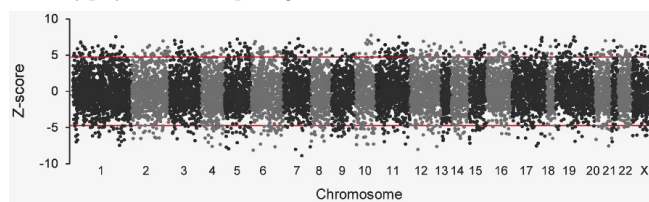


Fig 1 A graphic representation of the meta-analysis of gene expression of human glomeruli from DN+ compared to normal subjects. Meta-analysis was performed by Stouffer's Z-method. Z>0 shows up-regulation, and Z<0 shows down-regulation compared to healthy control. The red line indicates the genome-wide significant threshold of Z-value (± 4.73 , P -value = 2.2×10^{-6}). We identified 429 significantly up-regulated genes and 323 down-regulated genes in DN glomeruli ($P < 2.2 \times 10^{-6}$).

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Genome-wide association study identifies novel variants associated with diabetic kidney disease in Chinese

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Background and aims: Although several genetic variants have been identified to be associated with diabetic nephropathy, few genetic markers have been found to be able to predict development of kidney disease among subjects with type 2 diabetes (T2D). In this study, we utilized prospective samples from the Hong Kong Diabetes Registry to identify genetic predictors of diabetic kidney disease (DKD).

Materials and methods: All subjects had T2D and were free of DKD at time of enrolment and DNA collection. We identified 200 subjects who developed incident diabetic kidney disease, defined as T2D with estimated glomerular filtration rate < 60 ml/min per 1.73 m², with other causes of renal impairment excluded, during the follow-up period. Two hundred individuals who were age, gender and disease-duration matched who did not develop DKD were

identified from the registry as controls. We performed genome-wide association study using the Illumina 610Quad genotyping array. Validation was performed by de novo genotyping using a MassARRAY platform on additional samples from the Registry.

Results: After quality control on the SNP data, we analyzed a total of 446,343 SNPs. We identified 8 genomic regions which showed suggestive association with increased risk or protection against incident diabetic kidney disease ($p < 1 \times 10^{-5}$). De novo genotyping in additional validation samples of 850 incident DKD cases and 3064 controls free of DKD confirmed association of 2 novel variants with incident DKD. Cox regression analysis revealed that individuals who carry 1–2 risk variants at the two loci have increased risk of incident DKD with HR 1.502 (95% CI 1.25–1.81, $p = 1.58 \times 10^{-5}$).

Conclusion: We identified 2 novel variants associated with incident diabetic kidney disease in Chinese. Additional replication genotyping and targeted resequencing to identify the causal variants are currently underway.

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Glutathione peroxidase 1 (GPX1) variants, oxidative stress markers, and risk of kidney complications in patients with type 1 diabetes

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Background and aims: Glutathione peroxidase (GPX) is a class of antioxidant enzymes that catalyzes the reduction of hydrogen peroxide to water. GPX1 is the most abundant isoform and is expressed in all kidney cells. Advanced oxidation protein products (AOPP) and 8-iso-prostaglandine (isoprostane) were identified as markers of oxidative stress in patients with kidney disease. We investigated associations of GPX1 genotypes and plasma concentrations of AOPP and isoprostane with kidney complications in type 1 diabetic patients.

Materials and methods: Four SNPs in the GPX1 region were genotyped in SURGENE (n=340; 10-year follow-up), GENEDIAB (n=461) and GENESIS (n=498) cohorts of type 1 diabetic patients. Subsets of GENEDIAB (n=237) and GENESIS (n=393) participants were followed-up for 9 and 5 years, respectively. Plasma concentration of AOPP (spectrophotometry assay) and isoprostane (ELISA) were measured at baseline in 436 GENEDIAB participants. Hazard ratios (HR) or odds ratios (OR) were estimated for incidence and prevalence of kidney complications. $p \leq 0.02$ was significant.

Results: In SURGENE, the minor T-allele of rs3448 was associated with the incidence of microalbuminuria (HR 1.97, 95% CI 1.23–1.33, $p = 0.004$, n=76) and with the progression to a more severe stage of diabetic nephropathy (HR 1.91, 95% CI 1.26–3.02, $p = 0.002$, n=98). The variant was also associated with lower estimated GFR at baseline (78 ± 5 TT vs 93 ± 3 TC vs 87 ± 3 ml/min CC, ANCOVA, $p = 0.003$) and with faster decline of eGFR during follow-up (-8.49 ± 2.74 TT+TC vs -5.09 ± 2.67 ml/min.year-1 CC, $p = 0.01$; all SURGENE analyses adjusted for sex, age, duration of diabetes, diabetic retinopathy stages, and use of ACE inhibitors). In pooled analyses of GENESIS/GENEDIAB cohorts, the same variant was associated with the prevalence at baseline (OR 2.79, 95% CI 1.16–6.20, $p = 0.01$, n=82) and the incidence during follow-up of end stage renal disease (ESRD; HR 1.98, 95% CI, 1.38–2.92, $p = 0.0001$; n=51, analyses adjusted for the above mentioned covariates plus cohort membership). Association with the incidence of ESRD was also observed for rs9818758 and rs1987628. Plasma AOPP and isoprostane concentrations at baseline were higher in participants who developed ESRD during follow-up as compared to those who did not. The risk T-allele of rs3448 was associated with higher plasma AOPP (81 ± 5 TT+CT vs 64 ± 5 μ mol/l CC, $p = 0.02$) and isoprostane concentration (1.85 ± 0.08 TT+CT vs 1.57 ± 0.08 ng/ml CC, $p = 0.005$; ANCOVA adjusted for sex, age, duration of diabetes, eGFR and use of ACE inhibitors).

Conclusion: The minor T-allele of rs3448 was associated with kidney complications (incidence and progression of microalbuminuria, prevalence and incidence of ESRD, decline of eGFR) in patients with type 1 diabetes. The risk allele was associated with higher plasma concentration of markers of oxidative stress. Our results are consistent with the implication of GPX1 in the mechanism of renal protection against oxidative stress in patients with type 1 diabetes.

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Advanced glycation end products disrupt cholesterol feedback regulation in diabetic renal tubules

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Background and aims: Advanced glycation end products (AGEs) cause diabetic renal tubules damage in various ways. The aim of this study is to investigate the lipid-mediated injuries caused by AGEs in HK-2 cells and the renal tubules of type 2 diabetic rats.

Materials and methods: For the in vitro study, human renal tubular epithelial cell line (HK-2) was respectively incubated with Nε-(carboxymethyl) lysine (CML, a member of the AGEs family), CML+anti-RAGE (receptor of AGEs), low density lipoprotein (LDL), LDL+CML, LDL+CML+ anti-RAGE for 24h. At in vivo level, animal model was developed by high fat/sucrose diet feeding plus streptozotocin injection. Rats were divided into DM and DM+AG (Aminoguanidine, an inhibitor of AGEs) groups. The effects of cholesterol accumulation were examined by Oil Red O staining and a quantitative intracellular cholesterol assay. Serum CML was determined by HPLC-MS analyzer, and renal CML was measured by immunohistochemical staining. The urine protein and neutrophil gelatinase-associated lipocalin (u-NGAL) were measured by ELISA. The gene transcription and protein translation were analyzed by quantitative RT-PCR and western blot respectively.

Results: 1. CML increased Oil Red O staining and intracellular cholesterol ester in the absence or presence of LDL in HK-2 cells. Anti-RAGE reduced the lipid droplets accumulation. A strong staining of Oil Red O was also found in the renal tubules of the diabetic group, but less staining in the AG treated group. 2. CML upregulated both mRNA and protein expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), LDL receptor (LDLR), sterol regulatory element binding protein-2 (SREBP-2), and SREBP cleavage-activating protein (SCAP), which were inhibited by anti-RAGE. The overexpression of these molecules in the renal of the diabetic rats was also ameliorated by AG treatment. 3. AG reduced serum and renal CML. 4. The increased urine protein and u-NGAL were improved by AG.

Conclusion: For the in vitro study, we demonstrated CML increased lipid accumulation in HK-2 cells by disrupting the SCAP-mediated feedback regulation of HMG-CoAR and LDLR. On in vivo level, we also found that reduce of CML improved the disrupted SCAP-mediated feedback regulation in the renal tubules of type 2 diabetic rats. These data suggested that CML caused diabetic tubules injury might be via disturbing the intracellular feedback regulation of cholesterol. Inhibition of CML-induced lipid accumulation might be a potential renoprotective role in the progression of diabetic tubules damage.

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Inflammation and oxidative stress markers related to renal damage due to diabetes mellitus

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Background and aims: Currently, strategies to monitor renal damage in the course of Diabetes mellitus (DM) are focused on diabetes markers and renal function. Assuming that oxidative stress and inflammation are implicated in the pathophysiology of Diabetic Nephropathy (DN), the aim here was to assess markers of inflammation and oxidative stress in the course of a long-term model of DM and evaluate how different strategies of glycemic control would modulate these markers.

Materials and methods: An experimental model of streptozotocin-induced DM in male Wistar rats was employed here. After 12 weeks of diabetes induction, animals were treated with insulin alone or combined with metformin (100 mg/kg) or N-acetylcysteine (750 mg/kg) until a total period of 24 weeks. Non-diabetic or non-treated diabetic animals during 12 and 24 weeks served as control groups. Body weight and blood glucose were monitored weekly. HbA1c, urinary total protein and albumin, plasma IL-6 and TNF- α were assessed after different periods of diabetes. The same cytokines were quantified in renal tissue after 12 or 24 weeks, beyond plasma and kidney malondialdehyde (MDA) by HPLC-DAD.

Results: As expected, diabetic animals showed higher glycemic levels, water consumption and urine volume, and reduced weight gain compared

to non-diabetic. HbA1c was increased after 6, 12, 18 and 24 weeks of DM ($p<0.0001$). Renal function was significantly altered only from 12 weeks of DM, once diabetic animals presented higher proteinuria ($p=0.027$) and albuminuria ($p<0.0001$) compared to controls. At the end of 12 or 24 weeks, an enlarged kidney/body weight ratio was observed in non-treated diabetic groups ($p<0.0001$), indicating renal hypertrophy due to DM. All treatment schemes reverted these alterations after 18 and 24 weeks. In respect to inflammation markers, higher levels of plasmatic IL-6 were found in diabetic rats after 6 ($p=0.0015$) and 12 weeks ($p=0.0019$), but not in 18 and 24 weeks, showing this alteration is not persistent. NAC treatment had a pro-inflammatory effect, once a 3 fold increase of plasmatic IL-6 was observed ($p=0.0161$). No significant alteration of kidney IL-6 and TNF- α or plasmatic TNF- α were detected. On the other hand, local and systemic oxidative stress (MDA quantitation) were found after 12 and 24 weeks of DM ($p<0.0001$). Insulin alone or combined with metformin recovered this change in plasma and kidney, but NAC led to increased MDA levels, showing a pro-oxidant action ($p<0.0001$). Good Pearson or Spearman correlations were obtained between plasma MDA and glycaemia ($r=0.7554$, $p=0.0003$), HbA1c ($r=0.5790$, $p=0.0149$), proteinuria ($r=0.7495$, $p=0.0020$), relative kidney/body weight ratio ($r=0.6690$, $p=0.0024$) and kidney MDA ($r=0.5885$, $p=0.0102$).

Conclusion: Overall, therapeutic strategies employed here were capable to control changes related to DM, renal function and oxidative stress. NAC presented an unexpected pro-inflammatory and pro-oxidant effect. MDA can be considered a good oxidative stress biomarker related to renal damage in DM and could be used to improve therapeutic monitoring in DM.

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Predominant role of glomerular podocytes in mediating the deleterious effects of CB2 deficiency in experimental diabetic nephropathy

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Background and aims: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins and excessive extracellular matrix accumulation in the mesangium, resulting eventually in glomerulosclerosis and progressive renal impairment. A functionally active endocannabinoid system is present within the kidney. The cannabinoid receptor of type 2 (CB2) is expressed by both inflammatory cells and podocytes. CB2 receptor activation has beneficial effects in experimental DN, while CB2 deletion worsens both functional and structural abnormalities of DN confirming a protective role of signalling through CB2. To investigate the relative contributions of podocytes cells and monocytes to the phenotype of diabetic CB2^{-/-} mice, we have performed bone marrow (BM) transplantation experiments.

Materials and methods: Male CB2 knockout (CB2^{-/-}) or wild type (CB2^{+/+}) mice received bone marrow transplants at age 8 weeks. Twenty-four hours before transplantation, recipient CB2^{-/-} and CB2^{+/+} mice underwent whole body irradiation with 8 Gy. Post-irradiated CB2^{-/-} mice received a BM transplant from CB2^{+/+} mice (KO^{CWT}; $n=15$). Post-irradiated CB2^{+/+} mice received a BMT from CB2^{-/-} mice (WT^{CKO}; $n=15$). 4 weeks after BMT, diabetes was induced in all mice by intraperitoneal injection of streptozotocin (55 mg/kg) in citrate buffer delivered in 15 consecutive days. Control mice were injected with citrate buffer alone. 14 weeks after the induction of diabetes, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Urinary albumin excretion was measured by enzyme-linked immunosorbent assay. Expression of the slit-diaphragm protein, podocin, was assessed by immunofluorescence. Fibronectin mRNA levels were quantitated by real-time PCR on total renal cortex.

Results: Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated haemoglobin levels. BMT did not affect metabolic/physiological parameters in either CB2^{+/+} or CB2^{-/-} animals. Albuminuria was significantly ($p<0.05$) increased in the CB2^{+/+} diabetic animals [DM:234.0 (200.0–285.9) $\mu\text{g}/18\text{hrs}$, geometric mean (25th–75th percentile)] as compared to the controls [ND:68.1 (64.6–75.3)] and further enhanced by CB2 receptor deletion [DM CB2^{-/-}:359.9 (227.9–519.4); $p<0.05$ DM CB2^{+/+} vs DM CB2^{-/-}]. However, albuminuria was similar in diabetic CB2^{-/-} and diabetic CB2^{-/-} mice transplanted with bone marrow from CB2^{+/+} mice [DM-KO^{CWT}:376.8 (353.2–368.5)]. Moreover, in diabetic CB2^{+/+} animals, transplantation of CB2^{-/-} bone marrow did not affect the magnitude of albuminuria [DM-WT^{CKO}:236.2 (211.5–257.4)]. In the diabetic mice the increase in albuminuria was paralleled a significant reduction in podocin and this effect was further exacerbated in diabetic mice lacking CB2 receptors. However,

neither transplantation with BM from CB2^{+/+} mice in diabetic CB2^{-/-} animals, nor transplantation with BM from CB2^{-/-} mice into diabetic CB2^{+/+} animals altered podocin expression. Similarly, diabetes-induced upregulation of fibronectin expression was further exacerbated in CB2^{-/-} mice, but not altered by BMT.

Conclusion: These findings demonstrate that in experimental diabetes the BM-derived cells do not play a major role in mediating the deleterious effects of CB2 deficiency and suggests a predominant role of podocytes.

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Combinatorial therapy of cilostazol and probucol effectively reduces albuminuria through inhibition of proinflammatory pathways in podocytes

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Background and aims: Long-lasting inflammation occurring in glomeruli has been proved to be involved in the development of chronic kidney disease such as diabetic nephropathy (DN). The aim of this study is to clarify the mechanism of renoprotective effect of combinatorial therapy of cilostazol (CSZ) and probucol (PBC), both of which exhibit anti-inflammatory properties.

Materials and methods: We employed lipopolysaccharide (LPS) induced acute renal inflammation model. In brief, after treatment with CSZ, PBC alone or in combination, mice were intraperitoneally injected with LPS. After 24 h, urinary albumin levels were measured by ELISA and expression of MCP1 in glomeruli was analyzed by immunostaining. In vitro, we treated cultured podocytes with LPS in the presence or absence of CSZ, PBC individually or both and then estimated NF- κ B activation by immunostaining, MAPK phosphorylation by immunoblotting and reactive oxygen species (ROS) production by dihydroethidium staining. Also activation of NADPH oxidase (Nox) and components of Nox family were examined by lucigenin assay and real-time PCR, respectively.

Results: In mouse model, CSZ significantly reduced and PBC showed a tendency to decrease LPS-induced albuminuria and the concurrent administration showed a more potent effect. The MCP1 upregulation in glomeruli was suppressed by individual treatment of both drugs and more strongly by the combination. The MCP1 overexpressed by LPS was mainly produced in podocytes among the native cells of glomeruli. And the inhibitory effect of these drugs on MCP1 upregulation was shown in LPS-treated cultured podocytes similar to that in glomeruli. As for molecular mechanism, CSZ suppressed nuclear accumulation of NF- κ B and phosphorylation of p44/42 MAPK induced by LPS. Forskolin, an activator of adenylate cyclase, reduced p44/42 MAPK phosphorylation as well and a specific PKA inhibitor H89 reversed the inhibitory effect of CSZ, indicating CSZ suppresses p44/42 MAPK through cAMP-PKA activation. PBC also reduced LPS-induced nuclear accumulation of NF- κ B, while it failed to suppress p44/42 MAPK phosphorylation. PBC is originally developed as an anti-oxidant and it completely suppressed ROS production in response to LPS in both mouse glomeruli and cultured podocytes. Also, another anti-oxidative agent, NAC inhibited MCP1 upregulation and nuclear accumulation of NF- κ B, indicating that ROS generation is prerequisite for NF- κ B activation and following MCP1 induction. Further, PBC suppressed Nox activity and Nox4 expression, indicating PBC inhibits ROS generation through suppression of Nox4, which in turn lead to suppression of MCP1 upregulation induced by LPS.

Conclusion: We found the combination of CSZ and PBC had a strong protective effect on inflammatory renal dysfunction using LPS model. The combined effect of these drugs was due to the fact that CSZ and PBC showed anti-inflammatory effects through inhibition of different pathway, p44/42 MAPK and ROS, respectively. Although further investigations are needed, the combination therapy would hold promise for future refinement of treatment of CKD.

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High-fat meals induce systemic cytokine release without evidence of endotoxaemia-mediated cytokine production from circulating monocytes or myeloid dendritic cellsC.L. Fogarty¹, J.K. Nieminen², L. Peräneva¹, M.I. Lassenius¹, C. Forsbloom¹, P.-H. Groop¹, O. Vaarala², M. Lehto¹, FinnDiane Study Group;¹Folkhälsan Research Center,²National Institute for Health and Welfare, Helsinki, Finland.

Background and aims: Dietary fat is known to increase circulating levels of bacterial lipopolysaccharide (LPS) and proinflammatory cytokines in healthy subjects. Earlier studies have found increased intestinal permeability in patients with type 1 diabetes (T1D), suggesting that patients may display a more pronounced postprandial increase in circulating proinflammatory TLR4 agonists-LPS and triglycerides. While it has been long known that circulating inflammatory markers are predictors of progressive renal failure, we have recently shown that LPS activity levels are strongly associated with the components of the metabolic syndrome and the development of diabetic nephropathy in patients with T1D. Increased TLR4 responsiveness together with postprandially elevated circulating LPS and triglyceride levels may play a key role in the promotion and progression of inflammation, thereby increasing the risk of developing diabetic complications. The aim of the present study was to investigate the acute effects of two sequential high-fat meals on circulating cytokines and the LPS responsiveness of innate immune cells in the context of T1D. We hypothesized that high-fat diet-induced endotoxaemia would increase cytokine release from peripheral innate immune cells, which could in turn both induce systemic inflammation and modulate TLR4 responsiveness, particularly in patients with T1D.

Materials and methods: Eleven patients with T1D and eleven controls received two fatty meals: breakfast (8:00, 65 g fat) and lunch (12:00, 42 g fat). The patients were studied as part of the Finnish Diabetic Nephropathy (FinnDiane) Study. Fasting (8:00) and postprandial (14:00) blood samples were taken and incubated with or without *E. coli* LPS. Cytokine levels in serum, and cytokine responses in monocytes and mDCs were measured using flow cytometry

Results: At fasting, diabetic myeloid dendritic cells (mDCs) exhibited higher LPS-induced IL-6 and IL-1 β production than controls. Postprandially, patients with T1D and controls showed significant increases in the serum concentrations of eight inflammatory cytokines (IL-6, TNF- α , IL-1 β , IFN- α , IL-10, IFN- γ , IL-12, and MIP-1 β) without concomitant increase in serum LPS activity. Serum cytokine production was similar in both groups. No postprandial change was seen in the IL-6, TNF- α , or IL-1 β production of mDCs or monocytes.

Conclusion: Our results show that in whole blood, diabetic dendritic cells are hyperresponsive to *in vitro* LPS stimulation. Furthermore, in patients and controls alike, acute high-fat meals increase circulating cytokines but have no effect on serum LPS activity levels or cytokine production in circulating mDCs or monocytes. Our results suggest that postprandial increases in serum cytokine levels are neither mediated by circulating endotoxins nor the activation of circulating innate immune cells. The production of high-fat meal induced inflammatory markers is conserved between patients and controls and most likely regulated at the tissue level.

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and type IV collagen expression in human mesangial cells (HMCs) loaded with apo E2 remnant lipoproteins, and examined whether sitagliptin which increases incretin is effective to improve nephropathy in T2DM patients with apo E2 allele.

Materials and methods: Remnant lipoproteins were isolated from plasma of a patient with T2DM and apo E2/2 genotype by ultracentrifugal method. HMCs loaded with remnant lipoproteins were incubated for 24 h with GLP-1 at the concentration of 0, 50, 100 and 500 nmol/L. To evaluate the expression of TGF- β , type IV collagen and GLP-1 receptor mRNA in HMCs, RT-PCR procedure was performed. T2DM with apo E3/3 (n=38) and apo E3/2 genotype (n=7) were studied. Patients were treated with sitagliptin over 10 months. HbA1c, plasma TG and remnant cholesterol were compared before and after sitagliptin therapy. Remnant cholesterol was determined as RLP-cholesterol. Urinary albumin-to-creatinine ratio (ACR, normal range <30 mg/g) was measured as a marker of nephropathy.

Results: Apo E2 remnant lipoproteins significantly stimulated the expression of TGF- β and type IV collagen mRNA in HMCs. GLP-1 significantly suppressed the expression of TGF- β and type IV collagen mRNA in HMCs loaded with remnant lipoproteins. GLP-1 significantly stimulated the expression of GLP-1 receptor. In apo E3/3 patients, 10-month therapy with sitagliptin significantly reduced HbA1c (6.6 \rightarrow 6.0%), plasma TG (129 \rightarrow 107mg/dl) and remnant cholesterol (5.5 \rightarrow 3.9mg/dl), and ACR (57.4 \rightarrow 34.1mg/g). In apo E3/2 patients, it reduced HbA1c (6.8 \rightarrow 6.0), plasma TG (173 \rightarrow 119) and remnant cholesterol (8.0 \rightarrow 5.0), and ACR (119.0 \rightarrow 19.2). Reduction in HbA1c from baseline at 10 months was not significantly different between apo E3/3 and apo E3/2 patients, but reduction in plasma TG and remnant cholesterol, and ACR was significantly greater in apo E3/2 patients than in apo E3/3 patients.

Conclusion: GLP-1 suppresses the expression of TGF- β and type IV collagen in HMCs loaded with apo E2 remnant lipoproteins. Sitagliptin reduced plasma TG/remnant lipoproteins and ACR more greatly in apo E2 T2DM patients. It is suggested that incretin is effective to protect and improve diabetic nephropathy associated with apo E2 through its lowering effect of plasma TG/remnant lipoproteins and/or direct effect on mesangial cells.

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Effects of incretin on human mesangial cells loaded with apo E2 remnant lipoproteins and on nephropathy in diabetic patients

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Background and aims: In JDCS (Japan) and UKPDS 74, hypertriglyceridemia was detected as one of the risk factors for diabetic nephropathy. Increased remnant lipoproteins underlie hypertriglyceridemia. We first found that apo E2 is a genetic factor for diabetic nephropathy, and that increase of plasma TG/remnant lipoproteins caused by apo E2 contributes to nephropathy. Remnant lipoproteins are taken up by mesangial cells, and then cause renal damage. The frequency of apo E2 is approx.10% in Japan and western countries. Diabetic nephropathy is characterized by the accumulation of extracellular matrix protein in the glomerular mesangium. Incretin, which is widely used in T2DM patients, reduces plasma TG/remnant lipoproteins as well as glucose. There is little information about the effect of incretin on nephropathy. In the present study we examined the effect of GLP-1 on TGF- β

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Performance of creatinine and cystatin C based CKD-EPI equations to estimate glomerular filtration rate in type 2 diabetes

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Background and aims: Diabetes kidney disease (DKD) is the worldwide leading cause of end-stage renal disease. Current guidelines recommend annual screening with urinary albumin measurement and estimation of glomerular filtration rate (GFR) by equations. The aim of this study was to evaluate the accuracy of creatinine and cystatin C based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations to estimate GFR in patients with type 2 diabetes (DM) as compared to healthy adults.

Materials and methods: Cross-sectional study included healthy adults and DM patients with GFR higher than 60 mL/min/1.73 m². GFR was estimated by CKD-EPI creatinine (CKDEPIcreat), CKD-EPI cystatin C (CKDEPIcystC), and CKD-EPI creatinine-cystatin C (CKDEPI-CC) equations in comparison with GFR measured by a reference method (51Cr-EDTA). Each group (DM vs healthy) was stratified according to ages above and below 45 years and analyzed separately. Serum creatinine was measured by Jaffe traceable method (Advia 1800, Siemens Healthcare) and serum cystatin C was evaluated by immunoturbidimetric method (Dako, Cytomation). Accuracy (percentage of estimated GFR within 30% [P30] of measured GFR), bias (mean difference between measured and estimated GFR), and precision (standard deviation of bias) were assessed. Agreement was evaluated according to Bland & Altman analyses.

Results: One hundred healthy adult subjects and 84 DM patients, aged 38±14 (18-86) and 59±19 (31-82) years, respectively, were evaluated. Measured 51Cr-EDTA GFR was 112±19 (64-160) and 104±27 (60-184) mL/min/1.73 m², in healthy individuals and in DM patients, respectively. For the healthy group with less than 45 years of age, the measured 51Cr-EDTA GFR was 117±19 and the CKDEPIcreat, CKDEPI-CC and CKDEPIcystC were 117±13, 110±11 and 104±12 mL/min/1.73 m², respectively. Only CKDEPIcreat was in agreement with the reference method (P=0.894). In the DM group, the measured 51Cr-EDTA GFR (126±41 mL/min/1.73 m²) was different from all equations: CKDEPIcreat, CKDEPI-CC and CKDEPIcystC equations were, respectively 98±16, 95±16 and 93±18 mL/min/1.73 m². The Figure depicts the GFR mean values as well as the precision (SD) of the older individuals. In this set, the reference GFR method did not agree with any equation, in both groups. In healthy subjects older than 45 years, the accuracy (P30) was 94%, 91% and 85% for CKDEPIcreat, CKDEPI-CC and CKDEPIcystC, respectively. In DM patients older than 45 years, accuracy was 71%, 68% and 55% for CKDEPIcreat, CKDEPI-CC and CKDEPIcystC, respectively.

Conclusion: All CKD-EPI equations underestimated GFR in older adults, more markedly in type 2 DM patients. In younger subjects, the creatinine based equation presented the best performance.

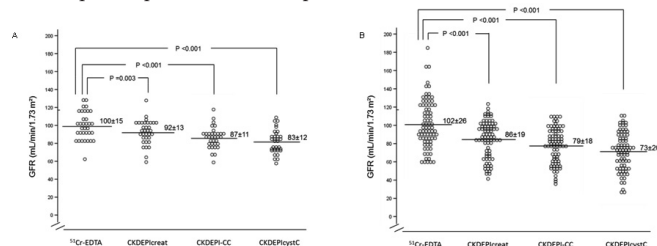


Figure – GFR values of individuals ≥ 45 years as measured by ⁵¹Cr-EDTA and by the CKD-EPI equations; healthy volunteers (N=34) in panel A and type 2 DM patients (N=79) in panel B.

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Albuminuria predicts progression to end-stage renal disease in diabetic patients

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Background and aims: Albuminuria in diabetic patients has been repeatedly shown to be a crucial risk factor for renal outcomes; however, previous studies examining these associations were limited to short observational period or surrogate markers including decline in GFR. There are few large cohort studies that observed patients until reaching end-stage renal disease. We conducted this study to clarify the association between albuminuria and hard renal endpoint in diabetic patients.

Materials and methods: This was a single-center historical cohort study consisting of adult Japanese diabetic patients who had both serum creatinine concentration and albuminuria data measured within 90 days at our hospital between 1995 and 2012, baseline estimated GFR (eGFR) ≥ 15 mL/min/1.73 m², and those who were observed at least 90 days. According to the baseline urinary albumin-to-creatinine ratio (ACR), patients were classified into the following 7 groups; <10, 10 to 29, 30-99, 100-299, 300-999, 1,000-2,999, and ≥ 3,000mg/g (Table). The primary endpoint was decrease in eGFR < 15mL/min/1.73 m². Cumulative incidence of the primary endpoint was estimated by the Kaplan-Meier method and the statistical differences among groups were examined by the log-rank test. HRs and the corresponding 95% CIs for reaching the endpoint were calculated using the Cox proportional hazard model.

Results: A total of 20,884 patients, 8,547 women and 12,337 men, with the mean age of 55 ± 14 (SD) years (range; 20-93 years) were studied; number of patients with the ACR group was listed in Table. During the median follow-up period of 5.6 years (up to 18.1 years), 1,033 patients reached the endpoint. Eighteen-year cumulative incidence of reaching the endpoint in each ACR group was summarized in the Table. Hazard ratio for patients in each ACR group after adjustment for gender, age and eGFR at baseline in reference to those with an ACR < 10 mg/g rose exponentially (Table, each p<0.001).

Conclusion: In this long-term large observational cohort study in Japanese diabetic patients, we found that stepwise rise in the risk of reaching ESRD according to increase in albuminuria. Higher levels of albuminuria, even within the normal range, may be associated with increased risk of renal hard endpoint.

Albuminuria (mg creatinine)	<10	10-29	30-99	100-299	300-999	1,000-2,999	≥ 3,000
No	9,432	5,362	2,787	1,356	1,008	622	317
18-year cumulative incidence of endpoint	1.9%	5.5%	14.1%	31.8%	71.8%	82.7%	98.1%
HR	Ref.	3.3	8.1	20.0	48.9	126.6	372.3

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Prevalence of albuminuria in subjects with impaired fasting glucose in Korean population

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Background and aims: We tried to assess the prevalence of albuminuria across the fasting plasma glucose (FPG) level including normal fasting glucose (NFG), impaired fasting glucose (IFG), and diabetes

Materials and methods: A total of 5,202 subjects who participated in the fifth Korea National Health and Nutrition Examination Survey (KNHANES V-2) were enrolled in this study. And, albumin-creatinine ratio was calculated.

Results: We divided subjects into five group according to fasting plasma glucose level of < 5.0 (NGT1, n=1905), 5.0-5.5 (NGT2, n=1784), 5.6-6.0 (IFG1, n=727), 6.1-6.9 (IFG2, n=268), and ≥ 7.0 (or diabetes, n=518) mmol/l. 7.6 percent (n=395). The prevalence of albuminuria in each group was 4.1, 6.0, 7.6, 12.3, and 23.4%, respectively, in the five groups (P < 0.01 for the trend). The prevalence of albuminuria in IFG2 was significantly higher than in IFG1 even after adjustment for age, sex, hypertension and obesity. In a multivariate logistic regression, independent associations with albuminuria were age, hypertension, and increasing fasting glucose including IFG2 and diabetes.

Conclusion: The current data suggest that prevalence of albuminuria may begin to increase in the higher range of IFG, independent of traditional risk factors.

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Arterial stiffness is more associated with albuminuria than decreased glomerular filtration rate in the development of type 2 diabetic nephropathy

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Background and aims: Arterial stiffness, a clinical atherosclerosis marker, was associated with the progression of chronic kidney disease (CKD) including diabetic nephropathy. Each albuminuria and decline of glomerular function rate (GFR) are being recognized as complementary rather than obligatory markers for diabetic nephropathy. The aim of study was to evaluate the association of arterial stiffness with albuminuria and/or GFR in the development of type 2 diabetic nephropathy.

Materials and methods: This study was an analysis from participants of The Relationship between Cardiovascular disease and Brachial-ankle Pulse Wave Velocity in Patients with Type 2 Diabetes (REBOUND) Study, which was a prospective multi-center cohort study recruited from 8 general hospitals in Busan, Korea. Type 2 diabetic patients aged 30 years and more were recruited and measured brachial-ankle pulse wave velocity (baPWV) as noninvasive marker for arterial stiffness. The patients were categorized into 4 groups according to albumin-to-creatinine ratio (ACR, normoalbuminuria and albuminuria) and eGFR ($< \geq 60$ mL/min/1.73 m²).

Results: A total of 2,613 patients were analyzed for this study. Maximal baPWV was significantly correlated with both ACR ($r = 0.302$, $P < 0.001$) and eGFR ($r = -0.218$, $P < 0.001$) in univariate analysis with whole subjects. After adjusting for age, sex, several significant clinical variables and eGFR, baPWV remained significant association with ACR in multivariate analysis ($r = 0.226$, $P < 0.001$). In patients with eGFR ≥ 60 mL/min/1.73 m² ($n = 2,134$), baPWV also showed positive correlations with ACR after adjusting in final model ($r = 0.219$, $P < 0.001$). However, baPWV lost significant correlation with eGFR after adjusting several significant clinical variables. In the patients with eGFR ≥ 60 mL/min/1.73 m², the increased level of baPWV remained significant association with to being albuminuria after adjusting for several clinical factors in multivariate model. In the normoalbuminuric patients ($n = 1,798$), the increased level of baPWV was not associated with decreased eGFR (< 60 mL/min/1.73 m², normoalbuminuric CKD) in multivariate model.

Conclusion: The results of this study suggest that arterial stiffness is more associated with albuminuria than GFR in development of type 2 diabetic nephropathy. Especially, arterial stiffness is not associated with normoalbuminuric CKD in type 2 diabetic patients.

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Sex difference in coronary artery calcification score and its use as a predictor of progression of diabetic nephropathy

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Background and aims: Cardiovascular disease (CVD) is a leading cause of death in type 2 diabetes mellitus (T2DM). The coronary artery calcification score (CACS) is believed to be one of the predictors of CVD. The progression of diabetic nephropathy (DN) leads to an increase in the risk of CVD and mortality. Moreover, it has been reported that progression of DN is strongly associated with macrovascular as well as microvascular complications. In this study, we assessed the relationship between progression of DN and CACS in T2DM patients at baseline and at 5-year follow-up.

Materials and methods: A total 103 T2DM patients (71 men, 65 ± 7 years old; 32 women, 67 ± 7 years old) of our medical center, who underwent multidetector CT scan and were assessed for DN each year, were studied. We excluded patients with a serum creatinine (Cr) over 1.0 mg/dl or those

who had iodine sensitivity. Patients were divided into two group based on median CACS derived from the Agatston score. Low CACS were those < 116 and < 65 for men and women, respectively, while high CACS were those ≥ 116 and ≥ 65 for men and women, respectively. The main measured outcome was the progression of DN stage defined by Japan Diabetes Society, American Diabetes Association, or European Association for the Study of Diabetes. Kaplan-Meier method and Cox proportional hazard analysis were performed to examine whether the relationship between CACS and DN progression was different between the sexes.

Results: At baseline, the male group with high CACS at baseline had a significantly higher albuminuria (283 ± 70 mg/g · Cr vs. 83 ± 39 mg/g · Cr, $p = 0.016$) and prevalence of diabetic retinopathy (DR, 53.1% vs. 21.9%, $p = 0.010$) than the male group with low CACS. Other baseline parameters (age, duration of DM, BP, HbA_{1c}, serum Cr, estimated GFR [eGFR], uric acid, lipid profile, white blood cell count, percentage of current smokers, use of statin, ACE inhibitor or angiotensin II receptor blocker) were not significantly different between the two groups. At baseline, the female group with high CACS were significantly older (70 ± 6 years vs. 64 ± 7 years, $p = 0.036$), had a lower level of eGFR (64.2 ± 10.6 mL/min/1.73² vs. 74.0 ± 11.7 mL/min/1.73², $p = 0.019$) and a higher level of Cr (0.70 ± 0.11 mg/dl vs. 0.63 ± 0.09 mg/dl, $p = 0.041$) compared with the female group with low CACS. Other parameters at baseline were not significantly different. At the 5-year follow-up, 52.8% of men in the high CACS group demonstrated progression of DN compared with 22.9% in the low CACS group ($p = 0.008$). In male patients without DR, those in high CACS group also had significantly higher progression of DN than those in the low CACS group (60.0% vs. 20.0%, $p = 0.007$). Patients with DR in the high and low CACS groups did not have a significantly different rate of DN progression (47.1% vs. 14.3%, $p = 0.145$). In women, DN progression was not significantly different between the high and low CACS groups (25.0% vs. 43.7%, $p = 0.233$) and the presence of DR had no effect on DN progression in the high and low CACS groups.

Conclusion: In men with T2DM, CACS is a good predictor of DN progression. DN progression was not related to the presence of DR, suggesting that the progression of nephropathy in men may be affected by atherosclerotic factors more than in women.

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Bimodal association of incidence and progression of diabetic nephropathy according to pack-years of smoking in type 1 diabetes

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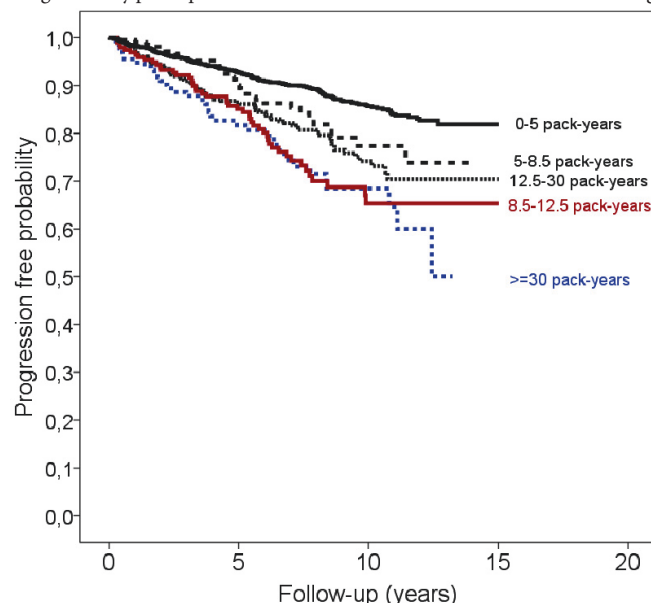
Background and aims: Our preliminary analyses of the relationship between dose of smoking as pack-years (py) and the initiation and progression of diabetic nephropathy (DN) based on the non-linear splines showed a bimodal distribution with the first peak at 10.5 pack-years and the second increase after 30 pack-years. This phenomenon was especially evident in women who progressed from normoalbuminuria to microalbuminuria.

Materials and methods: The study included 3,838 patients with type 1 diabetes. At baseline, mean age was 37.7 (IQR 28.8-47.0) years, duration of diabetes 21.4 (12.2-30.9) years, and 51% were males. Patients were followed for 6.3 (4.1-9.1) years. All patients with progressive DN were analyzed together; any progression from normo- to micro-, micro- to macro- or macroalbuminuria to end-stage renal disease. First the potential non-linear relationship between pack-years and progression of DN was modeled by Generalized Additive Models (GAM). The data were further analysed by Kaplan-Meier method and finally by Cox regression models adjusted for age, HbA_{1c}, systolic blood pressure (SBP) and triglycerides (TG) and providing hazard ratios (HR). Based on the bimodal distribution found by the GAM-analyses the data were divided into the groups 0-5, 5-8.5, 8.5-12.5 (± 2 years around the first peak), 12.5-30 and ≥ 30 pack-years (when risk started to increase again).

Results: There were a total of 438 progressors. Those who had smoked 8.5-12.5 py had a more favorable profile for clinical risk factors than those who had smoked ≥ 30 py (such as TG, SBP, age, duration of diabetes), although the risk of progression of DN was similar. The 10-year progression risks for the groups (0-5, 5-8.5, 8.5-12.5, 12.5-30 and ≥ 30 py) were 14.3% (95% CI 12.6-16.0), 22.6% (16.3-28.5), 34.7% (28.6-40.3), 25.8% (21.6-29.8) and 31.6% (24.7-37.8), respectively (Figure). HR was 2.2 (1.5-3.1) for those who had smoked 8.5-12.5 py, while it was 1.7 (1.1-2.7) for those who had smoked ≥ 30

py compared to those who had smoked 0–5 py. HR was 1.4 (0.9–2.1) and 1.3 (0.9–1.8) for those who had smoked 5–8.5 and 12.5–30 py.

Conclusion: Increase in pack-years of smoking is associated with progression of DN, but the relationship is not linear but rather bimodal. We conclude that the first peak might indicate an increased risk of a subset for the patients who are genetically predisposed and the second due to harmful effects of smoking.



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Reduction of microalbuminuria by calcium channel blockers in patients with type 2 diabetes mellitus and hypertension

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Background and aims: It has been suggested that reno-protective effects are different among calcium channel blockers. The aim of this study was to compare anti-albuminuric effect of different types of calcium channel blockers in patients with type 2 diabetes.

Materials and methods: A multicenter, randomized, open label, active-controlled study was conducted in seven hospitals in Korea. Inclusion criteria were age ≥ 18 years, hypertension treated with angiotensin receptor blocker or angiotensin-converting enzyme inhibitor, type 2 diabetes with HbA1c $\leq 8\%$, and persistent microalbuminuria. A total of 74 patients were randomized into the cilnidipine 10mg group ($n = 37$) or the amlodipine 5mg group ($n = 37$). Patients were assessed at baseline, 12 weeks and 24 weeks after treatment.

Results: Compared with cilnidipine group, amlodipine group showed greater diastolic blood pressure reduction at 24 weeks after treatment ($P=0.03$). In the cilnidipine group, urine albumin to creatinine ratio (ACR) was significantly reduced after 12 weeks (-53.0 ± 123.2 mg/g, $P<0.01$) and 24 weeks (-57.3 ± 106.9 mg/g, $P<0.01$) treatment. However, amlodipine group did not show any decrease in ACR after 12 weeks or 24 weeks treatment. Cilnidipine group showed significant reduction in ACR compared with amlodipine group after 12 weeks (-84.7 ± 106.8 mg/g in cilnidipine group and -9.5 ± 79.2 mg/g in amlodipine group, $P=0.02$) and 24 weeks (-84.0 ± 111.7 mg/g in cilnidipine group and 14.6 ± 119.4 mg/g in amlodipine group, $P<0.01$) treatment, particularly in patients with long duration of diabetes more than 10 years.

Conclusion: Cilnidipine shows greater microalbuminuria reduction than amlodipine in renin-angiotensin system blocker-treated hypertensive patients with long duration of type 2 diabetes.

Supported by: CKD PHARMA

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Adherence to medications improves risk reduction of end-stage renal disease in diabetes mellitus

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Background and aims: Diabetes is a prevalent chronic disease with significant complications. Uncontrolled glycemic is an independent risk factor and results in accelerated progression to end-stage renal disease (ESRD). To our knowledge, there is no study discuss the association between antihyperglycemic medication adherence and subsequent ESRD among individuals with type 2 diabetes. The specific aims of this study was to examine the effect of better adherence to antihyperglycemic therapy on preventing the risk of ESRD among newly diagnosed diabetic patients.

Materials and methods: This study used a representative cohort of 1 million people from National Health Insurance Research Database in Taiwan. All subjects aged 20 years or older who have been diagnosed with diabetes mellitus (ICD-9 code: 250.xx) and received newly treated with antihyperglycemic agents were included. Newly treated patients were identified as those patients who had not taken any antihyperglycemic agents before the first date of being diagnosed with diabetes. Adherence was a medication possession ratio (MPR) calculated by the sum of total days' supply of medication dispensed divided by the number of days (365) for each year equal or greater than 80%. ESRD was defined as patients received dialysis claims continuously for three months. Time-dependent medication adherence for estimating the risk of ESRD was applied in the Cox proportional hazard regression model. Covariates included age, gender, hypertension, ischemic heart disease, cerebrovascular disease, congestive heart failure, anemia, urinary tract infection, gout, chronic kidney disease, Charlson comorbidity index, use of statin, number of drugs therapy with hypertension, number of drugs therapy with diabetes and only use of metformin. All the analyses were performed using SAS version 9.3. The study was approved by the TMU-Joint Institutional Review Board.

Results: A total of 32,861 newly treated diabetes mellitus patients were enrolled and 327 cases developed ESRD during a mean follow-up of 4.9 years. The mean age was 56.4 ± 12.8 years, and male gender accounted for 53.6%. After adjustment for potential confounders, patients who were nonadherent to diabetes medications were associated with the increased risk of ESRD [hazard ratio (HR) = 1.56, 95% confidence interval (CI): 1.19–2.03]. In addition, results indicated that patients with hypertension (HR = 1.40, 95% CI = [1.04–1.87]), cerebrovascular disease (HR = 1.53, 95% CI = 1.05–2.24), chronic heart failure (HR = 3.89, 95% CI = 2.49–6.05), anemia (HR = 3.51, 95% CI = 2.06–5.97), chronic kidney disease (HR = 11.82, 95% CI = 8.61–16.22), monotherapy with hypertension (HR = 1.52, 95% CI = 1.06–2.17), use two or more therapy with hypertension (HR = 1.68, 95% CI = 1.09–2.59), and use two or more therapy with diabetes (HR = 1.56, 95% CI = 1.18–2.07) have higher risk of developing ESRD compared to their counterparts. However, patients only used metformin within the two year of diabetes diagnosis had lower risk of developing ESRD (HR = 0.23, 95% CI = 0.09–0.58).

Conclusion: This study showed nonadherence to antihyperglycemic medications are associated with increased the risk of ESRD among type 2 diabetes patients.

Clinical Trial Registration Number: 201204036

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Effect of glycaemic and serum electrolyte variation on cardiac electrical activity during haemodialysis in people with insulin treated diabetes

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Background and aims: One-year survival for diabetic patients on dialysis is 84.7% in the UK. Cardiovascular deaths account for 22%. Rapid electrolyte shifts, QT dispersion, left ventricular hypertrophy, myocardial structural

and functional abnormalities and sympathetic hyperactivity are potential causative factors. Hypoglycaemia has been linked to sudden death in insulin treated diabetes. To explore the relationships between; 1. glycaemic variation and heart rate, rhythm and QTc interval 4hrs before, during (3 to 4hrs) and 4hrs after haemodialysis (HD) 2. changes in serum electrolyte levels during HD and heart rate, rhythm and QTc interval.

Materials and methods: In our ongoing study, we undertook week-long continuous glucose monitoring and Holter monitoring, for 1 to 3 weeks in insulin deficient patients. Serum electrolytes and 12 lead ECGs were obtained at the beginning, middle and end of 3 HD sessions during the 1st study week. 10 insulin deficient patients (7 female, mean age 50.6±9.8 yrs) were monitored, during 72 HD sessions.

Results: Mean glucose levels during HD were significantly lower than pre-HD (8.4±3.6 vs 11.2±4.5mmol/L, $p<0.001$) and post-HD period (8.3±3.5 vs 12.3±4.7mmol/L, $p<0.001$) on paired samples. There were significant periods of hyperglycaemia (>13.0 mmol/L) during pre-HD (68.3±93mins vs 22.8±55mins, $p<0.005$) and also post-HD compared to HD period (89.5±82 vs 19.9±48.6mins, $p<0.001$) on paired samples, with no significant difference between pre and post-HD levels. Hypoglycaemia (<3.5 mmol/L) occurred more frequently during HD period, but was not statistically significant. Mean QTc interval was not significantly different between pre-HD and HD (420±20 vs 424±19ms, $p=0.2$), but was significantly prolonged during post-HD compared to pre-HD period (427±17 vs 420±20ms, $p<0.05$) on paired samples. Multiple short episodes of asymptomatic tachy- and brady-arrhythmias were noted in 6 out of 10 patients, including atrial tachycardia, non-sustained ventricular tachycardia, sinus bradycardia, ventricular bigeminy/trigeminy & junctional rhythm. Mean post-HD serum electrolytes were significantly lower than pre-HD levels ($n=30$): potassium (K^+) 3.3±0.3 vs 4.4±0.8 mmol/L ($p<0.001$), magnesium (Mg^{2+}) 0.78±0.07 vs 0.9±0.16mmol/L ($p<0.001$) corrected calcium (Ca^{2+}) 2.21±0.07 vs 2.27±0.14 mmol/L ($p<0.05$). A significant drop in K^+ and Mg^{2+} levels occurred in both first and second half of HD sessions. Ca^{2+} levels dropped significantly in first but not in second half of HD session. Post-HD QTc interval was significantly prolonged compared to pre-HD ECG (500±56 vs 471±44 ms, $p<0.005$). The significant prolongation occurred during second half of the HD session.

Conclusion: Glycaemic variation on HD days shows a 'U' shaped pattern with glucose levels being low during HD. There is significant prolongation of QTc interval during HD and subsequent period, which could be related to a drop in serum electrolyte levels. Potentially life threatening episodes of arrhythmias occur frequently in these patients. Larger study is required to understand the pathophysiological basis of these arrhythmias and their relation to glycaemia and electrolyte changes.

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1199

Improving quality: chronic kidney disease in diabetes

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Background and aims: Implementation of a continuous audit tool to facilitate focused review and optimal medicines management of those persons with diabetes and chronic kidney disease (CKD) stage 3 and above within the primary care setting. A significant event review within a general practice highlighted that when there is a decline in renal function outside a patient's formal diabetes review, medication adjustment is not always undertaken. A need was identified for the development of an audit tool to facilitate the continuous and focused review of CKD stage 3 and above within the practice diabetes population which in addition, would also enhance appropriate medicines management and safe prescribing.

Materials and methods: An automated weekly search engine using the practice information technology system was established to identify and invite in patients within three population sub groups (estimated glomerular filtration rates (ml/min/1.73m²) 46-60, 31-45, and <30), who were failing to meet practice established protocol targets to include optimal medication.

Results: Within the practice diabetes population of 616 patients, the prevalence of CKD stage 3 and above was 22% which equated to 135 patients. During the first six months, eight patients (6%) required adjustment of their diabetes medication therapy and four patients (3%) required alternative medication to their non-steroidal anti-inflammatory drugs. This demonstrated a move to enhanced medicines optimisation and safety. Other clinical interventions that are widely accepted as effective in delaying the onset or progression of CKD were also measured. Those patients without a recorded HbA1c fell from 8% to 3% and at the end of the six months all patients with

a HbA1c over 54mmol/mol had a review plan. Those without a blood pressure measurement fell from 3% to 2%. Patients who were not prescribed an angiotensin-converting enzyme inhibitor or angiotensin-II receptor antagonist where there was no contraindication, lowered from 6% to 1%. Those patients without a measurement of micro albuminuria rose from 12% to 13% thus highlighting an area for improvement. Three patients were identified as requiring referral to secondary care.

Conclusion: Against the background of an ever changing diabetes population and newer therapies, this audit tool, in combination with focused review, is effective in reducing variation and in improving quality of patient care. In addition, it affords timely intervention to ensure appropriate and safe medicines management within this complex patient population. It is hoped that the impacts will be increasingly evident over time, in terms of reduced patient mortality and morbidity.

PS 106 Clinical: hypertension in diabetes

1200

Is home blood pressure reporting in patients with type 2 diabetes reliable?

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Background and aims: Hypertension, as well as hyperglycemia, is well known as an important risk of cardiovascular and renal disease in patients with type 2 diabetes. Home blood pressure (HBP) monitoring is a valuable tool in the management of hypertension. To our knowledge no study has investigated the reliability of HBP measurements in patients with type 2 diabetes. The aim of this study was to evaluate the reliability of HBP reporting and determine the factors that affected it in patients with type 2 diabetes.

Materials and methods: A cross-sectional study was conducted in 280 consecutive patients with type 2 diabetes recruited from the outpatient clinic at our university. Patients were requested to measure triplicate morning and evening HBP using a digital automatic BP monitor and to record these measurements in a logbook over a 2-week period. The patients were not informed of the storage function of their BP monitor.

Results: Among a total of 280 patients, 155 (55.4%) were taking antihypertensive medication. The concordance rate between the self-reported data in the logbook and the stored data in the monitor was 78.6%. Although 65 of the patients (23.2%) had 100% “concordant data” that were matched the stored data in the monitor and 144 (51.4%) had more than 90% concordant data, 44 (15.7%) had 50% or less concordant data. The self-reported data were significantly lower than the stored data (mean of morning systolic BP: 129.8 ± 15.8 vs. 130.6 ± 16.2 mmHg, $p < 0.0001$). In addition, logbook BP was less variable than monitor BP (SD of morning systolic BP: 7.9 ± 3.2 vs. 9.8 ± 3.5 mmHg, $p < 0.0001$). The most frequent erroneous data (55.8%) were “selected data” that were randomly selected from multiple measurements in the monitor, the second most erroneous (23.3%) were “fictional data” that could not be found in the monitor, and the third most erroneous (11.6%) were “shifted data” that were stored in the monitor on another day but for the same time. The concordance rate correlated significantly with HbA1c ($r = -0.127$, $p = 0.0333$), HDL cholesterol ($r = 0.131$, $p = 0.0286$), triglycerides ($r = -0.121$, $p = 0.0429$), mean of morning systolic monitor BP ($r = -0.121$, $p = 0.0443$), mean of evening diastolic monitor BP ($r = -0.127$, $p = 0.0386$) and SD of morning systolic monitor BP ($r = -0.129$, $p = 0.0310$). Multiple regression analysis indicated that the independent explanatory variables concerning the concordance rate were HbA1c ($\beta = -0.156$, $p = 0.0149$) and current smoking ($\beta = -0.165$, $p = 0.0184$). Urinary albumin excretion also correlated with the SD of morning systolic BP in the monitor, but not with that in the logbook.

Conclusion: Patients with type 2 diabetes sometimes report erroneous HBP, especially those with poor glycemic control or a smoking habit. As a result of this inaccurate reporting, HBP control obtained from logbooks may appear better than that obtained from a monitor. A device capable of automatically saving BP data may be helpful for obtaining accurate measurements of HBP that will assist in providing optimal treatment for hypertensive patients with type 2 diabetes.

1201

Predictive value of markers of vascular damage for renal outcome in type 2 diabetes and essential hypertension

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Background and aims: We have shown that renal vasodilating response to nitrates (dynamic renal resistive index, DRIN) is an early vascular alterations of type 2 diabetes (T2DM) and essential hypertension (EH) already present in normoalbuminuric individuals. In this study we prospectively evaluated

whether this parameter and other markers of systemic vascular damage are able to predict microalbuminuria (MA) onset and renal function decline in these patients.

Materials and methods: We studied 65 individuals (27 T2DM and 38 EH) following them prospectively. Renal resistive index (RI), DRIN (% change in RI after glyceryl trinitrate, GTN 25 µg sl), endothelium-dependent (flow-mediated-dilation - FMD) and independent (response to GTN) vasodilation in the brachial artery, carotid-femoral pulse wave velocity (PWV), and augmentation index (AIx) were assessed. At the follow-up visit, MA onset was defined with urinary albumin-creatinine ratio (UACR) >30 mg/g; any reduction in estimated glomerular filtration rate (eGFR, CKD-EPI formula) was also considered as an endpoint.

Results: All patients were treatment-naïve at enrollment, whereas at the follow-up visit 62% were taking antihypertensive drugs and 37% were treated with anti-hyperglycemic agents. After a follow-up period of 4.1 ± 0.6 years, mean eGFR (CKD-EPI) decreased from 89.0 ± 14.4 to 86.4 ± 13.0 ml/min/1.73m², whereas UACR increased from 5 (0-29) to 9 (0-47) mg/g. According to our definition, 19 individuals developed MA and 12 a reduction in eGFR. At enrollment, patients who would develop MA tended to be older (60.4 ± 9.3 vs 53.8 ± 10.4 years, $p = 0.07$) and carrying more frequently diabetes than their counterparts. Among vascular parameters, RI (0.63 ± 0.04 vs 0.59 ± 0.06 , $p = 0.04$), DRIN (-6.4 ± 8.9 vs -10.2 ± 6.3 %, $p = 0.09$) and PWV (9.5 ± 1.3 vs 7.9 ± 1.5 m/s, $p = 0.003$), were worse at baseline in those who would develop MA during follow-up. Conversely, AIx (24 ± 11 vs 22 ± 13 %, $p = 0.93$), FMD (3.9 ± 1.9 vs 5.6 ± 3.9 %, $p = 0.27$) and GTN (5.1 ± 2.8 vs 5.6 ± 3.4 %, $p = 0.85$) were similar in the two groups. In T2DM patients there was a significant increase ($p < 0.05$) in RI (from 0.65 ± 0.05 to 0.68 ± 0.04) and PWV (from 8.7 ± 2.1 to 10.2 ± 2.0 m/s) during follow-up, while both were unchanged in EH patients (RI from 0.56 ± 0.03 to 0.59 ± 0.06 , PWV from 7.9 ± 1.5 to 8.3 ± 1.7 m/s). DRIN was not significantly modified during follow up. In T2DM, at enrollment DRIN (-2.2 ± 7.0 vs -7.5 ± 4.4 %, $p = 0.03$), but not RI (0.65 ± 0.4 vs 0.65 ± 0.05 , $p = 0.63$) or PWV (10.0 ± 1.6 vs 8.3 ± 2.4 m/s, $p = 0.14$), was significantly worse in patients who would develop MA, whereas in the EH group RI (0.60 ± 0.03 vs 0.57 ± 0.04 , $p = 0.04$) and PWV (8.7 ± 0.7 vs 7.8 ± 1.0 m/s, $p = 0.03$), but not DRIN (-11.6 ± 6.9 % vs -12.9 ± 7.6 %, $p = 0.62$), were altered in patients developing MA. At baseline, patient with reduction in eGFR were older and tended to have higher RI. Considering only T2DM patients, none of the explored vascular parameters were associated with reduction in eGFR.

Conclusion: These preliminary results suggest that some parameters of vascular damage like RI, PWV and DRIN are able to predict MA onset in EH and in T2DM, respectively. These markers of vascular damage might be useful in elucidating pathophysiology of renal damage and in predicting its development during the course of these chronic diseases.

1202

Annual deterioration of renal function in hypertensive patients with diabetes vs without diabetes

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Background and aims: Chronic renal disease (CKD) complicates hypertension and diabetes. Knowledge of the deterioration rate of CKD may anticipate adjustment of therapies with predominant renal elimination. We aim to evaluate the rate of annual deterioration of renal function in a large cohort of hypertensive patients either with diabetes (DM) and without it (non-DM) followed for 5 years in a reference out-patient hospital clinic of hypertension, and to relate it with BP and glycemic control.

Materials and methods: Out of a total of 1924 patients, 1023 patients (594 non-DM and 429 DM, 53% female, ageing 62.1 ± 10.2 years) were evaluated during the last 5 years for the annual evolution of renal function (MDRD) ambulatory 24-h blood pressure (ABP, SpaceLabs 90207) and metabolic parameters, corresponding to the analysis of 2378 patients-years.

Results: DM and non-DM did not differ for age (60.9 ± 10.1 v 62.8 ± 10.5 years), mean 24h BP levels ($134/86 \pm 12/10$ v $136/87 \pm 11/11$, nighttime $123/74 \pm 16/10$ v $122/73 \pm 15/10$ mm Hg), albuminuria (145 ± 430 vs 130 ± 370 mg/24h) and body mass index (28 ± 6 v 29 ± 8 kg/m²). DM v non-DM showed a higher (qui square $p < 0.01$) prevalence of stage 3 CKD (24.2% v 18.1%, GFR 30-59 mL/min/1.73m²), stage 4 (5.4% v 2.7%, GFR 15- 29) and stage 5 (0.8% v 0.5%, GFR 8.0 %). Each year net GFR was reduced by 3.3 ± 8.2 in DM vs 2.4 ± 7.7 mL/min/1.73m² in non-DM ($p = 0.12$, ns). In multivariate analysis, age, nighttime BP, the use of double inhibition of renin angiotensin system and HbA1C ≥ 8.0 % in DM were independent factors associated

with the deterioration of GFR. Also in average 16.2% of DM and 13.1% on non-DM moved each year towards the next and more severe stage of CKD ($p=0.051$). For initial GFR ≥ 90 mL/min/1.73m², 24% of DM and 18% of non-DM showed a reduction per year $\leq 10\%$ of the previous GFR value (qui square, $p=0.049$).

Conclusion: A progressive deterioration of renal function for each next year is frequent in diabetics and non-diabetics with hypertension. Beyond ageing, renal deterioration may be particularly dependent on BP control particularly at nighttime, on certain therapies and on highly abnormal glucose control.

1203

A systematic review and meta-analysis of the effects of diuretics and beta-blockers on glycaemic control in diabetes mellitus

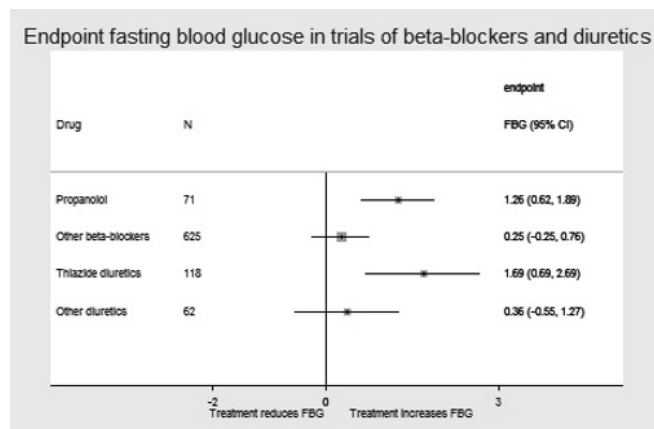
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Background and aims: Although there are reports that beta-adrenoceptor antagonists (beta-blockers) and diuretics can affect glycaemic control in people with diabetes mellitus, there is little information on how blood glucose concentrations are likely to change and by how much.

Materials and methods: We systematically reviewed the published literature to identify randomised controlled trials (RCTs) in which glycaemic control was studied in people with diabetes mellitus taking either beta-blockers or diuretics. We pooled end-point data on HbA1c and fasting blood glucose (FBG) concentrations using inverse variance fixed effects meta-analysis.

Results: We retrieved 3864 papers and found 10 studies (15 comparisons involving 1889 participants) of beta-blockers and 11 studies (12 comparisons involving 312 participants) of diuretics to include in the meta-analysis. One study included comparisons of both beta-blockers and diuretics, giving a total of 20 included trials. Beta-blockers increased fasting blood glucose concentrations by a mean of 0.64 mmol/l (95% CI 0.24 to 1.03) and HbA1c by 0.75% (95% CI 0.30 to 1.20), corresponding to 8.2 mmol/mol (95% CI 3.3 to 13.1) compared with placebo. Three trials (four comparisons) that studied the beta-blocker propranolol showed a larger increase in FBG concentrations than three trials that used other beta-blockers (1.26 mmol/l, 95% CI 0.62 to 1.89 compared with 0.25 mmol/l, 95% CI -0.25 to 0.76). Diuretics increased FBG by 0.96 mmol/l (95% CI 0.29 to 1.63) compared with placebo and HbA1c by 0.24% (95% CI -0.17 to 0.65), corresponding to 2.6 mmol/mol (95% CI -1.9 to 7.1) compared with placebo. The four trials (5 comparisons) that studied thiazide diuretics gave a larger increase in FBG levels than three trials that used non-thiazide diuretics (1.69 mmol/mol, 95% CI 0.69 to 2.69, and 0.36 mmol/mol, 95% CI -0.55 to 1.27 respectively), but when tested using meta-regression this difference was not significant ($p=0.101$). There were no significant differences in the numbers of hypoglycaemic events or other adverse events between beta-blockers and placebo in three trials.

Conclusion: In this synthesis of data we have quantified the effect of two commonly used types of antihypertensive medications on glycaemic control in diabetes. Both medication types increase fasting blood glucose and HbA1c concentrations in patients with diabetes by moderate but clinically significant amounts. Among beta-blockers, propranolol has the biggest effect, but there have been too few studies to allow a full analysis of other beta-blockers. These data will inform the monitoring and use of beta-blockers and diuretics in patients with diabetes.



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1204

Renoprotective effect of ACE-I and ARB in combination with hydrochlorothiazide in hypertensive type 2 diabetic patients

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Background and aims: To compare the effects on albumin excretion rate (AER) of two combined preparations: ramipril/hydrochlorothiazide (Ram/htz) and valsartan/hydrochlorothiazide (Val/htz)

Materials and methods: This is a 12 months randomized parallel group study. 130 patients with hypertension (140 mmHg \leq SBP $<$ 180 mmHg and DBP $<$ 110), controlled Type 2 diabetes and albuminuria ≥ 20 and $<$ 500 μ g/min were randomized to Ram/htz (group 1, $n=61$) or to Val/htz (group 2, $n=69$). A dose up-titration from Ram 2.5/htz 12.5 mg up to Ram 5/htz 25 mg or from Val 80/htz 12.5 mg up to Val 160/htz 12.5 mg was allowed up to W12 to achieve blood pressure (BP) targets of SBP $<$ 140 mmHg and DBP $<$ 90 mmHg. The mean urinary albumin excretion was evaluated on overnight urine collections. All patients completed 12 months of follow-up. Followings parameters were measured: blood pressure (BP), BMI and HbA1c

Results: 130 patients in 2 groups were similar in terms of sex, age (58.2 vs 58.4), diabetes duration (10.8 vs 11.1), BMI, BP (systolic 156 vs 155.8 mmHg, diastolic 93.3 vs 92.8 mmHg), HbA1c (7.9 vs 8.0 %), cholesterol, creatinine and smoking habits. At the end of 1 year period: systolic BP 156 ± 11.2 mmHg vs 142 ± 12.5 for group 1 ($p < 0.01$) and 155.8 ± 11.1 mmHg vs 143 ± 14.2 mmHg for group 2 ($p < 0.01$) and diastolic blood pressure 93.3 ± 6 mmHg vs 84.5 ± 9.2 mmHg for group 1 ($p < 0.01$) and 92.8 ± 6.9 mmHg vs 85.0 ± 7.6 mmHg for group 2 ($p < 0.01$). Pre and post treatment AER were 75.3 ± 25.5 μ g/min vs 48.5 ± 21 μ g/min ($p < 0.01$) for group 1 and 76.1 ± 26.1 μ g/min vs 49.0 ± 23.4 μ g/min ($p < 0.01$) for group 2. The other parameters remained unchanged in both groups

Conclusion: Treatment with such combined preparations as ramipril/hydrochlorothiazide and valsartan/hydrochlorothiazide were equally effective in reducing AER as well as hypertension in hypertensive type 2 diabetic patients

1205

Effect of weight loss on abnormal 24-h blood pressure patterns and endothelial function in hypertensive obese patients with and without type 2 diabetes

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Background and aims: Nocturnal hypertension [HT, night systolic blood pressure (SBP) $>$ 120/70 mmHg], non-dipper status (nocturnal BP fall $<$ 10% of daytime values), high pulse pressure (PP, difference between 24-h SBP and DBP readings) and endothelial dysfunction are associated with an increased risk of cardiovascular disease. Our aim was to compare the 1-year effect of significant weight loss on abnormal 24-h BP patterns (nocturnal HT, PP and non-dipper status) and endothelial function in severely obese hypertensive patients with and without type 2 diabetes mellitus (DM).

Materials and methods: Patients with documented HT undergoing bariatric surgery (BS) were studied and evaluated before BS and 12 months post-operatively. Antihypertensive treatment was withdrawn one week before each evaluation. Anthropometric data were collected, BP (24-h ambulatory BP measurement) was measured and endothelial function was determined by endothelial-dependent flow-mediated vasodilatation (FMD) of the brachial artery.

Results: Thirty-three patients [12 with DM and 21 patients without DM] were studied. DM remission was observed in 75% and HT remission was observed in 67% and 86% of patients with and without DM. The table below shows the main results obtained.

Conclusion: In patients with HT surgery-induced weight loss was associated with a sizeable decline in BP with a high HT remission range. After weight loss patients with DM maintain a high prevalence of abnormal 24-h BP patterns and continue to have a high cardiovascular risk. In addition, endothelial function did not improve.

n	Type 2 DM (yes)		Type 2 DM (no)	
	12		21	
	Baseline	12 m	Baseline	12 m
Age (year)	58 (5)*		50 (10)	
Hypertension duration (year)	10 (8)*		4 (4)	
Δ Body mass index (kg/m ²)		13 (5)		15 (5)
Δ Waist circumference (cms)		26 (11)		27 (13)
Δ Systolic BP (mmHg)		14 (9)		20 (2)
Δ Diastolic BP (mmHg)		5 (7)		8 (8)
Non-dipper status (%)	54	57	46	61
Nocturnal Hypertension (%)	57	67*	44	22
Pulse pressure (mmHg)	63 (7)	54 (8)*	60 (8)	44 (6)
FMD (%)	10 (8)	10 (6)	9 (6)	8 (6)

*p < 0.05 compared patients with and without type 2 DM; Δ 12 months – basal; BP: blood pressure

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1206

Associations of visceral fat area and HOMA-R with both diabetes mellitus and hypertension in a health checkup programme: NingenDock H. Hirose^{1,2}, M. Takayama², Y. Iwao², H. Kawabe¹, Y. Sugino²;

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Background and aims: Although the visceral fat area (VFA) value and homeostasis model assessment insulin resistance index (HOMA-R) in patients with metabolic syndrome (MetS) or type 2 diabetes mellitus (DM) are known to be higher, the relationship of VFA and HOMA-R with hypertension (HT) remains unclear. In the present study, we investigated the relationship of blood pressure status with VFA, subcutaneous fat area (SFA), HOMA-R, and insulin secretion capacity (HOMA-β) in male subjects during an annual health check-up program.

Materials and methods: A total of 1737 Japanese male subjects, aged 45-74 years, who underwent the whole body health check-up program 'Ningen-Dock' between August 2012 and October 2013 were enrolled in this study. Informed consent was obtained from each subject, and 9 subjects were excluded owing to predetermined exclusion criteria. The subjects were divided into 4 groups according to glucose tolerance status: normal glucose tolerance (NGT), preDM, DM, and DM with medication (DM+Tx), and blood pressure status: normotensive (NT), high normal (HN), HT, and HT with medication (HT+Tx). Both VFA and SFA were measured at the umbilical level using computed tomography.

Results: VFA (mean±SD) in the PreDM (n=425), DM (n=120), and DM+Tx (n=175) groups were significantly higher than that of the NGT (n=1008) group (117±43, 133±54, and 124±54 vs. 101±44cm², F=31.6 and P< 0.0001 by ANOVA). Furthermore, the VFA of the HN (n=231), HT (n=202), and HT+Tx (n=517) groups were significantly higher than that of the NT (n=778) group (109±43, 118±46, and 128±49 vs. 95±42cm², F=58.8 and P< 0.0001). The HOMA-R (median [Q1-Q3]) of the HN, HT, and HT+Tx groups were also significantly higher than that of NT group (1.4 [1.0-1.9], 1.5 [1.0-2.1] and 1.6 [1.1-2.7] vs. 1.2 [0.8-1.8], F=38.0 and P< 0.0001). Multiple logistic regression (MLR) analysis of DM as a dependent variable revealed that low HOMA-β, high HOMA-R, age, VFA, and smoking were relevant. Moreover, MLR analysis of HT as a dependent variable revealed that age, VFA, alcohol consumption (more than 20g ethanol/day), BMI, and HOMA-R were relevant (Table 1).

Conclusion: These results suggest that VFA and HOMA-R correlate with HT, as well as type 2 DM and MetS, in Japanese male subjects aged 45-74 years.

Table 1. Multiple logistic regression analysis of hypertension as a dependent variable in 1728 male subjects, aged 45 to 74 years

No.	Variables	β	SE(β)	z	P	Odds ratio	95% CI
		-8.00	1.18				
1	Age (per 10)	0.54	0.07	7.56	<0.0001	1.72	1.49 ~ 1.98
2	VFA (per 50)	0.35	0.08	4.56	<0.0001	1.42	1.22 ~ 1.64
3	Alcohol	0.43	0.11	4.00	0.0001	1.54	1.25 ~ 1.90
4	BMI (per 5)	0.45	0.13	3.56	0.0004	1.57	1.23 ~ 2.01
5	log[HOMA-R]	0.84	0.24	3.49	0.0005	2.32	1.44 ~ 3.71
	DM	0.28	0.14	1.94	0.0520	1.69	1.23 ~ 2.34
	AUC = 0.714						

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1207

Endoscopic, duodenal-jejunal bypass liner in obese patients with or without type 2 diabetes lowers incidence of metabolic syndrome and improves cardiovascular risk

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Background and aims: The endoscopically delivered duodenal-jejunal bypass liner (DJBL) exhibits robust metabolic effects in obese subjects with or without type 2 diabetes (T2D). Several clinical studies have been conducted in development of the DJBL. We report metabolic and cardiovascular (CV) effects of DJBL in obese subjects with or without T2D across 2 uncontrolled clinical studies.

Materials and methods: Forty subjects completed the intended 12 month treatment period (baseline mean ± SEM: age 44.5 ± 1.85 yr, BMI 45.2 ± 1.09 kg/m², A1C 8.6 ± 0.48% and 6.2 ± 0.26% in subjects with [n=13] and without [n=27] T2D, respectively, 77.5 % females). Most common co-morbid conditions were hypertension (47.5%), GERD/Gastritis (12.5% each), and hyperlipidemia (10.0%).

Results: At baseline 35/40 (87.5%) subject had metabolic syndrome (as defined by ATPIII criteria) and, using the FraminghamBMI, Framingham Lipid, and UKPDS CV risk engine models, the 10-year CV risk was determined to be 11.9 ± 2.09%, 9.0 ± 1.52%, and 5.9 ± 0.93%, respectively. After 12 months of intended implant time, the overall cohort lost 18.6 % in total body weight on average and 17.7 cm from the waist. Additionally, systolic BP dropped by -7.6 mmHg, LDL-cholesterol decreased by 0.6 mmol/L, and A1c reduction of 2.1 % in the diabetic subgroup. The improvement in CV risk factors translated to a 20-32% reduction in 10-year CV risk (see table) and a 37% decreased in subjects meeting the metabolic syndrome criteria, (20/40 subjects (p<0.001). Analysis of concomitant medications during the 12 month period showed trends for dose reductions or discontinuation of glucose, BP, and lipid-lowering agents in 87% of subjects. Adverse events were mostly of gastrointestinal nature, mild or moderate in severity, and tended to diminish in frequency in the weeks after initial device implantation.

Conclusion: In 40 obese subjects with or without T2D who completed 12 months of DJBL implantation, clinically meaningful improvements in prevalence of metabolic syndrome and 10-year CV risk level regardless of the model used. The device appears to be generally safe and well-tolerated. This non-surgical approach warrants further characterization as an important treatment option to improve CV risk in obese patients with or without T2D.

Risk Engine	Baseline* (%)	12 Months* (%)	% Change*	P-value
Framingham—BMI	11.9 ± 2.09	9.5 ± 1.79	-19.2 ± 4.18	0.0003
Framingham—Lipid	9.0 ± 1.52	7.0 ± 1.32	-25.0 ± 4.18	<0.0001
UKPDS	5.9 ± 0.93	3.9 ± 0.59	-32.5 ± 3.28	<0.0001

*Mean ± SEM

Clinical Trial Registration Number: NCT00791128; NCT00985491

Supported by: GI Dynamics sponsored clinical studies

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Multifactorial risk factor control in clinical practice and risk of cardiovascular disease in type 2 diabetes: report from the Swedish national diabetes register

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Background and aims: Cardiovascular complications are still the major cause of morbidity and mortality in patients with type 2 diabetes (T2D). To lower this risk, guidelines advocate multifactorial risk factor control with focus on glucose control, blood pressure (BP) and blood lipids levels. The aim of this observational study was to assess the effect of change in these risk factors on risk for cardiovascular disease (CVD) and mortality in T2D, also in a subgroup with albuminuria.

Materials and methods: 47,020 T2D patients from the Swedish National Diabetes Register, aged 30–70 years, were followed from baseline visits 2003–2006 until 31 Dec 2009, mean follow-up 5.7 years. In all, mean age 60±8 years and diabetes duration 7±6 years. Reference group (n= 13,005) with stable values or increase in HbA1c, systolic BP (SBP) and the ratio non-HDL-to-HDL-cholesterol (non-HDL:HDL) during the study period was compared with seven groups (n=3766 to n=6509) of decrease in either each or in combinations of these risk factors. The reference group with stable or increasing risk factor values had mean HbA1c increasing from 52 to 57 mmol/mol, mean SBP increasing from 135 to 141 mmHg, and mean non-HDL:HDL increasing from 2.9 to 3.3. Groups with decrease in HbA1c showed decrease from mean 60 to 52 mmol/mol, with decrease in SBP showed decrease from 146 to 130 mmHg and with decrease in non-HDL:HDL had decrease from 3.3 to 2.5.

Results: Compared with reference group hazard ratios (HR) with 95% confidence intervals at Cox regression for fatal/nonfatal CVD were 0.45 (0.40–0.52) with decrease in HbA1c only, 0.31 (0.27–0.36) with non-HDL:HDL, 0.27 (0.23–0.31) with SBP, 0.25 (0.22–0.29)–0.36 (0.31–0.41) with decrease in two of three risk factors combined, and 0.24 (0.21–0.28) with all three risk factors decreasing, all p<0.001. Corresponding HR for total mortality were 0.36 with HbA1c decrease only, 0.23 with non-HDL:HDL, 0.27 with SBP, 0.23–0.36 with decrease in two risk factors, and finally 0.29 with all three risk factors decreasing, p<0.001 after adjusting for clinical characteristics, CVD risk factors, treatments and a history of CVD. Similar results were found in a subgroup of 10,004 patients with albuminuria at baseline, HRs for fatal/nonfatal CVD 0.27–0.49 with decrease in one, 0.26–0.36 with decrease in two risk factors, and 0.23 with all three risk factors decreasing, all p<0.001, while corresponding HR for total mortality were 0.22–0.31, 0.19–0.30, and 0.25, p<0.001.

Conclusion: Successful risk factor control in patients with T2D in clinical practice with reductions in HbA1c, blood pressure and blood lipids was associated with 55–76% lower risk for CVD and 36–77% lower risk for total mortality.

1209

Depressive symptoms predict incident stroke independent from low physical activity in older patients: the Japanese elderly diabetes intervention trial (J-EDIT)

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Background and aims: Although recent remarkable advances in treatment of diabetes have been made, conventional risk factors cannot fully explain the increased risk of stroke in older patients with diabetes mellitus. Psychological factors and physical inactivity have been considered to be one of new potential risk factors for micro- and macrovascular complications in diabetes mellitus. Diabetes mellitus is more likely to have depressive symptoms, which lead to low physical activity. In contrast, physical inactivity is associated with depression. The aim of the study is to examine whether depressive symptoms predict incident stroke independent from conventional risk factors and low physical activity in a 5-year follow-up study of elderly patients with diabetes mellitus.

Materials and methods: The Japanese Elderly Diabetes Intervention Study (J-EDIT) is a randomized, controlled, multi-centre, prospective intervention trial using a total of 1173 older patients (aged ≥65 years) with type 2 diabetes mellitus from 39 institutions. Nine hundred and seven patients were used for analysis after excluding 110 patients who were dropped out very early or had missing data about depressive symptoms at baseline. Mean age was 71.8 years. Depressive symptoms were evaluated using a short form of the Geriatric Depression Scale (GDS-15, 15 items). Physical activities were assessed using the Baecke questionnaire. Cox regression analysis was performed to examine the independent association between depressive symptoms, conventional risk factors, or physical activity, and incident stroke.

Results: During the 5-year of follow-up, 50 non-fatal and fatal strokes occurred. The score of GDS-15 was 4.6 ± 3.0 at baseline. The prevalence of those who had GDS-15 ≥8 was 12.7%. The incidence of stroke in the patients who had the GDS-15 ≥8 was significantly higher than those who had the GDS-15 <8 (P<0.001, log-rank test). Depressive symptoms (GDS-15 ≥8) predicted incident stroke after adjusting for age, sex, HbA1c, systolic blood pressures, non-HDL-C, and HDL-C (HR=3.2, 95%CI: 1.7–6.2). After adding physical activity to the model, the association between depressive symptoms and incident stroke remained significant (HR=3.2, 95%CI: 1.6–6.3, p<0.001).

Conclusion: Depressive mood is one of independent predictors for stroke in older patients with diabetes mellitus.

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Fasting triglyceride is an independent cardiovascular risk factor in HbA_{1c} less than 6.5%

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Background and aims: Although LDL is the primary target for lipid-lowering therapy and non-HDL cholesterol is a secondary target in patients with elevated triglyceride (TG) levels, triglyceride rich lipoprotein (TGRLP) is still an early, reliable, and practical predictor for vascular inflammation. However, the independent relationship of triglycerides to the risk of future CVD events has long been controversial. More commonly identified are milder triglyceride level elevations (ie, 100 to 500 mg/dl) associated with environmental triggers such as poor diet, lack of exercise, obesity, DM, and metabolic syndrome (Mets). The goal of our study tried to find whether TG less or more than 100 mg/dl could be the predictive factor for cardiovascular inflammation.

Materials and methods: A total of 6883 subjects (TG < 100 mg/dl, N=3639, mean age 51.4 ± 11.9 ; TG ≥ 100 mg/dl, N=3244, mean age 52.6 ± 11.2 ; p < 0.01), whose HbA_{1c} was less than 6.5 %, without history of type 2 DM or documented cardiovascular diseases were enrolled in this retrospective case control cohort study. Levels of various lipid fractions and C-reactive protein (CRP) were measured.

Results: Data revealed as: (1) Total CHOL 198.2 ± 32.3 mg/dl versus 208.9 ± 34.4 mg/dl, in TG < 100 mg/dl & TG ≥ 100 mg/dl (p < 0.01). (2) HDL-C 54.9 ± 12.6 mg/dl versus 46.6 ± 10.1 mg/dl, in TG < 100 mg/dl & TG ≥ 100 mg/dl (p < 0.01). (3) LDL-C 111.4 ± 28.6 mg/dl versus 124.8 ± 30.7 mg/dl, in TG < 100 mg/dl & TG ≥ 100 mg/dl (p < 0.01). (4) CRP 0.154 ± 0.425 mg/dl versus 0.185 ± 0.442 mg/dl, in TG < 100 mg/dl & TG ≥ 100 mg/dl (p < 0.01). Linear regression for TG < 50 mg/dl, strong correlation with LDL-C, HDL-C, total CHOL (p < 0.01), but not for CRP (p=0.5637). On the other hand, strong correlation in linear regression was noted between TG ≥ 100 mg/dl and HDL-C (p < 0.01), total CHOL (p < 0.01), CRP (p=0.0237), but not for LDL-C (p=0.0542). On the basis of CRP comparison, CRP is statistically correlated with subjects with TG ≥ 100 mg/dl, but not for subjects TG < 100 mg/dl.

Conclusion: Our results may suggest that healthy persons with HbA_{1c} less than 6.5 % but fasting TG ≥ 100 mg/dl could be the predictor for cardiovascular inflammation, independent from the level of LDL-C. An optimal triglyceride cut point is intended to define one physiological parameter of cardio-metabolic health in the future.

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Lipoprotein(a), type 2 diabetes and vascular risk in angiographed coronary patients

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Background and aims: Lipoprotein (a) [Lp(a)] especially in young individuals is an important cardiovascular risk factor. However, data on the long-term vascular risk conferred by Lp(a) in patients with type 2 diabetes (T2DM) are scarce.

Materials and methods: Lp(a) was measured in a cohort of 909 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease; vascular events were recorded over 10 years.

Results: Median Lp(a) at baseline was significantly lower in patients with T2DM (n=260) than in subjects without T2DM (10 [interquartile range 1–34] vs. 16 [1–54] mg/dl; p=0.017). Prospectively, 27.8% of our patients suffered vascular events. Lp(a) proved to be a strong and independent predictor of vascular events in total population with a standardized adjusted hazard ratio (HR) of 1.15 [1.03–1.27]; p=0.006 as well as in subjects without T2DM (HR 1.22 [1.10–1.36]; p<0.001) but not in patients with T2DM (HR 0.990 [0.79–1.22]; p=0.888). An interaction term T2DM x Lp(a) was significant (p<0.001), indicating that Lp(a) was a significantly stronger predictor of vascular events in subjects without T2DM than in patients with T2DM.

Conclusion: Lp(a) in patients with T2DM is low and is not associated with the incidence of vascular events. The power of Lp(a) as a predictor of cardiovascular events is significantly modulated by the presence T2DM.

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Impact of age on the cardiovascular event risk conferred by HbA_{1c} in patients with established coronary artery disease

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Background and aims: In the present study we tested the hypothesis that age modulates the impact of HbA_{1c} on cardiovascular event risk in patients with established coronary artery disease (CAD).

Materials and methods: We prospectively recorded cardiovascular events over a mean follow-up period of 4.4±1.2 years in a large consecutive series of 816 patients with angiographically proven CAD, including 376 subjects <65 years and 440 subjects ≥65 years.

Results: During follow-up, the incidence of cardiovascular events was 9.3% among subjects <65 years and 24.8% among subjects ≥65 years (p<0.001). Among the younger patients, HbA_{1c} strongly and significantly predicted cardiovascular events (HR 1.54 [1.06–2.23]; p=0.022), but not among the older patients (HR 1.22 [0.94–1.59]; p=0.125). An interaction term age x HbA_{1c} was statistically significant (p=0.007), indicating that HbA_{1c} was a significantly stronger predictor of cardiovascular events among younger than among older CAD patients.

Conclusion: We conclude that HbA_{1c} is a significantly stronger predictor of cardiovascular events in younger patients than in older patients with established CAD.

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All-cause mortality in type 2 diabetes in New Zealand: development and validation of a risk model

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Background and aims: Type 2 diabetes is associated with a substantial increase in the risk of death. This study aimed to derive an up-to-date predictive risk model for all-cause mortality in type 2 diabetes in our multi-ethnic population.

Materials and methods: We used primary care data from a large national multi-ethnic cohort of patients with type 2 diabetes in New Zealand (the NZ Diabetes Cohort Study) and linked these with national death records to develop several predictive risk models for 5-year risk of mortality. We then validated these models using information from a separate cohort of patients with type 2 diabetes.

Results: 26,864 people, mean age 62 years, were included in the development cohort with a median follow up time of 9.1 years and 6333 deaths (24%). We developed three models initially using demographic information and then adding progressively more readily available clinical detail. The final model, which also included markers of renal disease (albuminuria and estimated GFR), proved to give best prediction of all-cause mortality with a C-statistic of 0.80 in the development cohort and 0.79 in the validation cohort (7,610 people, follow-up 5 years, 759 deaths, 10%); it was well calibrated. In addition to the usual known risk factors including HbA_{1c}, ethnicity was a major influence with hazard ratios of 1.37 for Maori, 0.79 for Pacific Peoples, 0.41 for East Asian and 0.55 for Indo-Asian compared with European (all p<0.001).

Conclusion: We have developed a model that provides an accurate assessment of patients' risk of death using information normally available in primary care. While our results are broadly similar to models previously published from smaller cohorts in other countries, they are superior to most and apply to a wide range of patients of multiple ethnicities and over recent years since 2000. Reasons for the major influence of ethnicity, both adverse and protective, are as yet unclear.

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Years of life lost attributable to type 2 diabetes in Germany

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Background and aims: In Europe a decline in the diabetes-related mortality has been observed in recent years. However, in comparison to the general population, people with type 2 diabetes still had a reduced life expectancy. Years of life lost (YLL) is an important epidemiological measure to quantify the burden of a chronic disease in a population. YLL attributable to type 2 diabetes has never been reported for Germany.

Materials and methods: We calculated the sex-specific YLL attributable to type 2 diabetes for a birth cohort in Germany (born 1955). The resulting YLL is a population average, independent from the individual life-time diabetes risk. The underlying incidence and mortality rates of known and unknown diabetes stem from the KORA S4/F4 survey, a cohort study with baseline examinations in 1999–2001 and follow-up period until 11/2009. Mortality rates of the general population (including a projection for future years) stem from the German Federal Statistical Office. Since KORA data have a restricted age range, we used data from the Danish diabetes register to expand the age range. Beside an analytical calculation of YLL based on a comparison of the areas under the survival curves, we additionally used a micro-simulation to assess statistical uncertainty. Calendar time trends in the incidence of diabetes and mortality rates of persons with diabetes are not known for Germany and are assessed by scenarios. The scenarios assume a change of +/-1 and +/-2% per year for the incidence and a change of -2 and -1% in case of the mortality in the diabetic population.

Results: The results are shown in Table 1. In the base case, i.e. no annual trends, the average YLL in the cohort is 5.7 and 2.4 years for men and women, respectively. Results from the simulation and the analytical calculation agree well within the confidence bounds. The uncertainty about trends in diabetes incidence and mortality in the diabetic population exceeds the statistical uncertainties in these estimates. In the male and female subgroup of the cohort, the range of YLL in the scenarios is 2.2–9.9 and 0.6–3.9 years, respectively. Independent from the scenario, it is striking how big the difference between men and women is. The reason lies in the different age courses of the prevalence in men and women. The prevalence in men is higher than in women until the age of about 80.

Conclusion: On population average, presence of type 2 diabetes was associated with a loss of 5.7 life years in men and of 2.4 years in women of the 1955 birth cohort, respectively. The uncertainty about the trends in incidence and mortality leads to uncertainty in the estimated YLL. Due to the high numbers of persons born in Germany in 1955, the total YLL attributable to type 2 diabetes sums up to more than 1.5 million years in the best case, 4.6 million years base case, and 7.5 million years in the worst case. These numbers refer

to the 1955 birth cohort alone. Therefore, despite improved diagnosis of diabetes and better medical treatment, type 2 diabetes is still associated with an enormous reduction of life-time.

Incidence	Mortality	Method	YLL males (years)	YLL females (years)
KORA	KORA	Simulation	5.9 (5.3 – 6.5)	2.5 (2.0 – 2.9)
KORA	KORA	Analytical	5.7	2.4
KORA – 2%	KORA	Analytical	3.8	1.7
KORA – 1%	KORA	Analytical	4.5	2.0
KORA	KORA – 2%	Analytical	2.2	0.6
KORA	KORA – 1%	Analytical	4.1	1.1
KORA + 1%	KORA	Analytical	7.3	3.0
KORA + 2%	KORA	Analytical	9.9	3.9

Table 1: Sex-specific years of life lost (YLL) in the different incidence and mortality scenarios. The YLL in the simulation (first row) is given with 95% confidence intervals (in brackets).

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Predictors of ischaemic heart disease and cerebrovascular attack in late elderly diabetic individuals: lessons from 9.1 years study of 4014 diabetic patients

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Background and aims: High LDL-cholesterol (LDL-C) and glucose levels are risk factors for ischemic heart disease (IHD) in middle-aged diabetic individuals; however, the risk among the elderly, especially late elderly older than 75y.o., is not well known. The aim of this study was to identify factors that predict IHD and cerebrovascular attack (CVA) in the elderly and to investigate their differences by age, especially legend effect.

Materials and methods: We performed a prospective cohort study (Japan Cholesterol and Diabetes Mellitus Study) with 9.1 years of follow-up. A total of 4,014 patients with type 2 diabetes and without previous IHD or CVA (1,936 women; age 67.4±9.5 years, median 70 years; <65 years old, n=1,261; 65 to 74 years old, n=1,731; and ≥ 75 years old, n=1,016) were recruited on a consecutive outpatient basis from 40 hospitals throughout Japan. Lipids, glucose, and other factors related to IHD or CVA risk, such as blood pressure (BP), were investigated using the multivariate Cox hazard model.

Results: Two hundred eighteen cases of IHD and 138 CVAs (7.8 and 5.7/1,000 people per year, respectively) occurred over 8.8 years. Systolic blood pressure on registration was correlated with IHD in all patients (hazard ratio (HR):1.012, P<0.05). LDL-cholesterol (LDL-C) on registration was correlated with IHD in patients >75 years old (HR:1.014, P<0.01). In contrast, fasting plasma glucose, HDL-C and age were correlated with CVA in all subjects (P<0.05) and HDL-C was correlated with CVA in all three generations (75 years old). Fasting plasma glucose and glycated hemoglobin (HbA1C) was correlated with CVA in patients of 65 to 74 years old. Age was correlated in patients >75 years old. Kaplan-Meier estimator curves showed same tendency, however, laboratory data on registration (legend effect) and those just before events (IHD and CVA) affects differently.

Conclusion: IHD and CVA in late elderly diabetic patients were predicted by LDL-C or HDL-C. LDL-C, fasting blood glucose, HbA1C and SBP may have the effect for atherosclerotic events (IHD or CVA) as legend effect, age dependently. These age-dependent differences in risk are important for developing individualized strategies to prevent atherosclerotic disease.

Clinical Trial Registration Number: UMIN-CTR, UMIN00000516

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Silent, advanced coronary artery disease in type 1 diabetes of forty years duration is prevalent and associated with glycaemic control

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Background and aims: The natural history and the pathophysiology of atherosclerotic disease in type 1 diabetes is not known in detail. We therefore studied in detail the progression of atherosclerosis in a uniquely characterized cohort of type 1 diabetes with a long duration of the disease and defined factors associated with the progression of atherosclerosis in this population.

Materials and methods: The Oslo study cohort originally started in 1982. Forty-five individuals with type 1 diabetes were randomized and included. In 1999 a subpopulation of 29 participants, not significantly different from the total group, without signs or symptoms of coronary heart disease were evaluated for coronary artery disease with exercise ECG, quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS). Twenty-six of these participants were enrolled for the same examination in 2009 (mean age 53 (7)). Twenty-three of these had technical adequate IVUS pullbacks for analysis. By QCA a vessel stenosis >50% of lumen diameter were classified as significant. By IVUS, an intimal thickness above 0.3mm was considered significant. % vessel area stenosis was defined as plaque area divided by vessel area x100.

Results: The QCA and IVUS results from 1999 and 2009 are reported in table 1. A total of 3264 arterial segments in 63 vessels were evaluated with IVUS in this last follow-up. In 1999 ten of the patients had mild atherosclerotic disease (% vessel area stenosis <20) whereas all had progressed to moderate to severe atherosclerosis examined by IVUS (% vessel area stenosis >20%) in 2009. We observed that HbA1c and LDL cholesterol levels ten years before this last follow-up were associated with progression of % vessel area stenosis, β 0.1 (0.01-0.2), $p=0.030$ and β 0.12 (0.3-0.33), $p=0.0013$, respectively.

Conclusion: Our data show no over-all progression of severe atherosclerosis evaluated by QCA. The plaque thickness was not significantly changed over ten years. However, the IVUS examinations revealed a progression of moderate to advanced atherosclerosis over ten years in type 1 diabetes patients of forty years disease duration. This despite that many of the participants used statins for many years. One of the reasons for the discrepancy in QCA and IVUS may be that remodelling in the coronary vessels underestimates the stenosis on QCA. Our data show the need to evaluate coronary atherosclerosis thoroughly in long term type 1 diabetes and reconfirm that LDL and blood glucose through many years may be main drivers of the process. Our findings are novel as there are no other studies with longitudinal data of the atherosclerotic process evaluated by QCA and IVUS in long term type 1 diabetes with silent macrovascular disease.

Table 1: Comparison of the QCA and IVUS data from 1999 and 2009 in type 1 diabetes patients.

	1999 n= 29	2009 n=26 (23)
Pathological exercise ECG	15 %	17 %
QCA, >50 % stenosis	34 %	26 %
IVUS, intima thickness >0.3 mm	72.2 %	85.2 %
IVUS (mean plaque thickness)	0.56 (0.033)	0.66 (0.26)

The data are presented as % and mean (SD).

Supported by: South-Eastern Norway Health authority, Oslo Diabetes research centre

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Impact of gender on the risk of coronary atherosclerosis and cardiovascular events conferred by HbA_{1c} in subjects without known diabetesA. Muendlein¹, C.H. Saely^{2,3}, A. Vonbank², D. Zanolin^{1,3}, P. Rein², H. Drexel^{2,4}¹VIVIT Institute, Feldkirch, Austria, ²Academic Teaching Hospital Feldkirch, Austria, ³Private University of the Principality of Liechtenstein, Triesen, Liechtenstein, ⁴Drexel University College of Medicine, Philadelphia, USA.

Background and aims: Diabetes confers a larger increase in the relative risk of cardiovascular events among women than among men. Whether gender also affects the association of HbA_{1c} with coronary atherosclerosis and cardiovascular events among subjects without known diabetes is unknown.

Materials and methods: We enrolled a large consecutive series of 1479 patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD), including 495 women and 984 men who did not have previously known diabetes. Significant CAD was diagnosed in the presence of significant coronary stenoses $\geq 50\%$. Prospectively, we recorded cardiovascular events over 4.4 ± 1.2 years.

Results: Among women, 36.4%, 56.2%, and 7.4% and among men 44.2%, 46.6%, and 9.1% had HbA_{1c} values of $<5.7\%$ (normal according to ADA criteria), 5.7–6.4% (at risk of diabetes according to ADA criteria), and $\geq 6.5\%$ (diabetes according to ADA criteria), respectively. The prevalence of angiographically diagnosed significant CAD in these HbA_{1c} categories was 31.2%, 38.2%, and 47.2% among women (ptrend = 0.041) and 63.2%, 65.3% and 64.8% among men (ptrend = 0.589). An interaction term gender x HbA_{1c} was statistically significant ($p < 0.001$), indicating that the association of HbA_{1c} with CAD was significantly stronger among women than among men. During follow-up, the incidence of cardiovascular events was 21.5% in women and 28.5% in men ($p = 0.002$). Among women, HbA_{1c} strongly and significantly predicted cardiovascular events (adjusted OR for a 1% increase in HbA_{1c} HR 2.08 [1.24–3.03]; $p < 0.001$), but not among men (HR 1.12 [0.94–1.53]; $p = 0.145$). An interaction term gender x HbA_{1c} again was statistically significant ($p = 0.011$), indicating that HbA_{1c} was a significantly stronger predictor of cardiovascular events among women than among men.

Conclusion: We conclude that gender significantly modulates the risk of coronary atherosclerosis and cardiovascular events conferred by HbA_{1c} in subjects without known diabetes.

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Diabetes is not a coronary artery disease risk equivalent among womenH. Drexel^{1,2}, C.H. Saely^{1,3}, A. Vonbank¹, D. Zanolin^{4,3}, P. Rein¹¹Academic Teaching Hospital Feldkirch, Austria, ²Drexel University College of Medicine, Philadelphia, USA, ³Private University of the Principality of Liechtenstein, Triesen, Liechtenstein, ⁴VIVIT Institute, Feldkirch, Austria.

Background and aims: Diabetes *per se* is widely considered a coronary artery disease (CAD) risk equivalent, particularly among women. We aimed at investigating the contribution of baseline coronary atherosclerosis to the risk of diabetic women for future vascular events in a prospective cohort study on subjects who were characterized by coronary angiography at baseline.

Materials and methods: Vascular events were recorded over 10 years in 598 consecutive women undergoing coronary angiography for the evaluation of established or suspected stable CAD.

Results: From our women, 271 had neither type 2 diabetes (T2DM) nor significant CAD (i.e. coronary stenoses $\geq 50\%$) at the baseline angiography, 79 had T2DM but not significant CAD, 152 did not have T2DM but had significant CAD, and 96 had both T2DM and significant CAD. Non-diabetic women without significant CAD had an event rate of 12.5%. The event rate was similar in T2DM women without significant CAD (15.2%; $p = 0.749$), but higher in non-diabetic women with significant CAD (32.9%; $p < 0.001$). Women with both T2DM and significant CAD had the highest event rate (43.8%; $p < 0.001$). Importantly, T2DM women without significant CAD had a significantly lower event rate than non-diabetic women with significant CAD ($p = 0.003$).

Conclusion: We conclude that T2DM *per se* is not a CAD risk equivalent among women. Moderate-risk diabetic women without significant CAD and very high-risk diabetic women with significant CAD add up to a grand total of high-risk diabetic women. This is why diabetes seems to be a CAD risk equivalent in many epidemiological studies.

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Comparison of renal function markers for risk prediction in subjects with type 2 diabetes (ZODIAC-47)A.E. Timmer^{1,2}, I.J. Riphagen^{1,2}, I. Drion¹, A. Alkhalaf¹, G.W.D. Landman¹, K.H. Groenier^{1,2}, G. Navis³, H.J.G. Bilo^{1,3}, N. Kleefstra^{1,4}, S.J.L. Bakker³¹Diabetes Centre, Isala Clinics, Zwolle, ²Departments of Internal Medicine and General Practice, UMCG, ³Department of Internal Medicine, UMCG, ⁴Department of Internal Medicine, UMCG, Groningen, Netherlands.

Background and aims: Advanced chronic kidney disease, one of the complications in type 2 diabetes, is a well known predictor for cardiovascular (CV) disease and mortality. Since measurement of glomerular filtration rate (GFR) is complex, clinical laboratories currently report an estimated GFR based on serum creatinine (SCr) measurement. Cystatin C was suggested to more accurately reflect kidney function and to improve risk prediction for the risk of death. However, it is not known whether other renal markers such as urea, uric acid and potassium improve risk prediction of CV and all-cause mortality. Our aim was to compare the predictive performance of these markers in type 2 diabetes.

Materials and methods: Patients with DM2 participating in the Zwolle Out-patient Project Integrating Available Care (ZODIAC) study were included. Cox regression analyses were used to investigate the associations of the renal markers with CV and all-cause mortality. Our multivariable model consisted of CV risk factors including age, sex, BMI, smoking, systolic blood pressure, total cholesterol-to-HDL ratio, duration of diabetes, HbA_{1c}, use of RAAS-inhibitors, history of CV diseases and log albumin-to-creatinine ratio. Harrell's C statistics were used to compare the predictive performance of the markers.

Results: We included 1,185 patients (46% male, age 67 ± 12 years). Mean plasma concentrations of SCr, cystatin C, urea, uric acid and potassium were $95 \pm 21 \mu\text{mol/L}$, $1.0 \pm 0.3 \text{ mg/L}$, $6.7 \pm 2.3 \text{ mmol/L}$, $0.33 \pm 0.09 \text{ mmol/L}$ and $4.4 \pm 0.4 \text{ mmol/L}$, respectively. After median follow-up for 5.5 [IQR 3.1–10.1] years, 354 patients died (30%), with 149 deaths (13%) attributable to CV causes. Univariable associations of the markers (per SD increase) with CV and all-cause mortality are shown in Table 1. In univariable Cox regression analyses, all markers were significantly associated with CV and all-cause mortality, with the highest C statistics for cystatin C. If renal markers were added to multivariable models, all renal markers except potassium remained independent predictors for CV and all-cause mortality, but the added value in terms of increase in C-statistic was limited and insignificant for all markers.

Conclusion: In type 2 diabetes, we found that cystatin C was a better predictor of CV and all-cause mortality than SCr, urea, uric acid and potassium. After adjustment for known CV risk factors, there was no added value in predictive performance for any of the studied markers.

	Cardiovascular Mortality		All-Cause Mortality	
	HR (95% CI)	Harrell's C	HR (95% CI)	Harrell's C
Serum Creatinine	1.57 (1.44–1.73)	0.67	1.46 (1.36–1.58)	0.61
Cystatin C	1.64 (1.50–1.78)	0.72	1.62 (1.52–1.72)	0.72
Urea	1.57 (1.42–1.74)	0.61	1.53 (1.42–1.65)	0.61
Uric Acid	1.57 (1.35–1.82)	0.60	1.35 (1.21–1.51)	0.57
Potassium	1.17 (1.02–1.35)	0.54	1.16 (1.05–1.27)	0.54

Supported by: GSK, Gentian

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Impaired kidney function is a diabetes risk equivalent in patients with established coronary artery diseaseH. Winkler¹, C.H. Saely^{2,3}, D. Zanolin^{1,3}, A. Vonbank², P. Rein², H. Drexel^{1,2,4}¹VIVIT Institute, Feldkirch, Austria, ²Academic Teaching Hospital, Feldkirch, Austria, ³Private University of the Principality of Liechtenstein, Triesen, Liechtenstein, ⁴Drexel University College of Medicine, Philadelphia, USA.

Background and aims: Type 2 diabetes (T2DM) is a paramount risk factor for cardiovascular disease, in particular among patients with established coronary artery disease (CAD). Similarly, chronic kidney disease (CKD) confers a high risk of cardiovascular events. We aimed at investigating the single and joint effects of T2DM and of CKD on cardiovascular risk in patients with angiographically proven CAD.

Materials and methods: We prospectively recorded cardiovascular events over 10 years in a cohort of 1423 patients with angiographically proven CAD.

CKD was defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m².

Results: The risk of cardiovascular events was significantly higher in T2DM patients (n=171) than in non-diabetic subjects (39.1% vs. 28.7%; $p < 0.001$) and also was higher in patients with CKD (n=116) compared to those with an eGFR ≥ 60 ml/min/1.73 m² (47.2% vs. 28.7%; $p < 0.001$). When both, T2DM and CKD were considered, 841 subjects had neither T2DM nor CKD, 336 had T2DM but not CKD, 145 did not have diabetes but had CKD, and 101 had both diabetes and CKD. When compared with the event rate among patients with neither T2DM nor CKD (26.3%), event rates were significantly higher in patients with T2DM who did not have CKD (34.8%; $p=0.007$) and in non-diabetic patients with CKD (42.8%; $p=0.020$) and were highest in patients with both, T2DM and CKD (53.5%; $p < 0.001$). Further, patients with both, T2DM and CKD were at a significantly higher event risk than those with T2DM but no CKD ($p=0.011$) and those without T2DM but with CKD ($p=0.048$). Event rates were similar in patients with T2DM but not CKD and in non-diabetic patients with CKD ($p=0.798$).

Conclusion: We here report the novel findings that CKD and T2DM contribute synergistically to cardiovascular event risk and that CKD is a T2DM risk equivalent in patients with established coronary artery disease.

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Associations between BMI, HbA_{1c} and mortality among people with screen-detected type 2 diabetes. ADDITION-Denmark

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Background and aims: The associations between HbA_{1c}, BMI, cardiovascular morbidity and all-cause mortality have earlier been investigated among people with type 2 diabetes, but not in the same study and previous results have been divergent. The obesity paradox implies greater survival among people with chronic diseases who are overweight or slightly obese compared to normal weight individuals. Considering this, we aim to examine whether BMI among people with screen-detected type 2 diabetes modify the associations between 1) HbA_{1c} and cardiovascular morbidity and 2) HbA_{1c} and all-cause mortality.

Materials and methods: This is a cohort study including 1533 patients aged 40–69 years with type 2 diabetes identified through the Danish arm of the Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-detected Diabetes in Primary Care (ADDITION-DK). At baseline, HbA_{1c}, BMI and cardiovascular risk factors were measured and information on medication, lifestyle and socioeconomic status were provided by means of a questionnaire. At follow-up, information on death or discharge from a Danish hospital were obtained from national registers. Information on BMI were available for 1489 patients. Five groups were formed according to BMI. Hazard ratios for the associations between baseline HbA_{1c}, cardiovascular morbidity and all-cause mortality will be estimated in each group using Cox multivariate regression model. The results will be adjusted for sex, age, medication, lifestyle, socioeconomic status and comorbidity.

Results: Baseline characteristics are shown in table 1. Preliminary results indicate that among people with screen-detected type 2 diabetes those with BMI ≥ 35 tend to be younger than those with BMI <35. The percentage of current smokers and the consumption of alcohol seem to be higher among people with BMI <30 compared to people with BMI ≥ 30 . Interestingly the proportions of people with HbA_{1c} >7% appear to be equally distributed among the five BMI groups and likewise there are no immediate differences in systolic blood pressure and levels of cholesterol.

Conclusion: Full results and conclusions concerning the associations between HbA_{1c}, cardiovascular morbidity and all-cause mortality will be presented at EASD Annual Meeting 2014. Our data indicate that a high BMI increases the risk of diabetes as individuals in the heaviest group tend to have developed diabetes at a younger age. Overall our full results will play a part in optimizing treatment strategies by clarifying the basis of rational treatment. Importantly our results will also contribute to an overview of influential factors regarding prognosis for people with type 2 diabetes. Such identi-

fication may help general practitioners to direct attention to those with the poorest prognosis and consequently act at an earlier stage. This per se could reduce or delay complications to diabetes.

	BMI groups					All
	18.5 ≤ BMI < 25	25 ≤ BMI < 27.5	27.5 ≤ BMI < 30	30 ≤ BMI < 35	BMI ≥ 35	
n	165	258	291	491	280	1489
(%)	(10.8)	(16.8)	(19.0)	(32.0)	(18.3)	(100.0)
Age at diagnosis, median (Q1; Q3)	62.3 (56.9; 65.9)	61.8 (55.9; 65.0)	60.9 (55.9; 65.0)	60.9 (55.4; 64.6)	59.1 (53.4; 63.1)	60.8 (55.4; 64.7)
Male sex, n (%)	73 (44.2)	169 (65.5)	201 (69.1)	282 (57.4)	122 (43.6)	848 (57.0)
Current smoker %	43.9	33.2	35.5	31.1	31.6	34.0
Alcohol consumption (units of alcohol/week), median (Q1; Q3)	7.5 (1.3; 19.7)	6 (3.2; 17.1)	8 (4.0; 16.8)	5 (1.4; 17.9)	3 (0.4; 15.6)	6 (1.9; 17.3)
Systolic blood pressure (mmHg), mean (95% CI)	147.7 (144.1; 151.3)	147.7 (145.2; 150.1)	150.7 (148.3; 153.0)	148.0 (146.4; 149.7)	148.4 (145.8; 151.0)	148.5 (147.4; 149.5)
HbA _{1c} (%), median (Q1; Q3)	6.2 (5.6; 7.8)	6.3 (5.8; 8.1)	6.3 (5.7; 7.4)	6.4 (5.9; 7.7)	6.5 (6.0; 7.8)	6.4 (5.8; 7.7)
- HbA _{1c} < 7%, %	77.6	69.8	80.1	73.9	70.7	74.2
- HbA _{1c} > 7%, %	22.4	30.2	19.9	26.1	29.3	25.8
Total cholesterol (mmol/l), mean (95% CI)	5.6 (5.4; 5.8)	5.8 (5.6; 5.9)	5.7 (5.5; 5.8)	5.6 (5.5; 5.7)	5.6 (5.5; 5.8)	5.7 (5.6; 5.7)

Table 1: Baseline characteristics according to BMI groups
Q1 = 1. quartile, Q3 = 3. quartile

Clinical Trial Registration Number: NCT00237549

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Development of a prediction model for fatal and non-fatal coronary heart disease and cardiovascular disease in patients with newly-diagnosed type 2 diabetes mellitus

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Background and aims: To construct a model for predicting CHD, and cardiovascular disease (CVD) risk in newly-diagnosed type 2 diabetic patients in a southern European region. External validation of two other cardiovascular risk models and internal validation of our developed model were assessed.

Materials and methods: A 10-year prospective population-based cohort study was performed with 777 newly-diagnosed type 2 diabetic patients older than 24 years in a Sentinel Practice Network. Cardiovascular risk factors, CVD events, and mortality were registered. The patients without CVD at baseline (659/777) who had data available for all variables (605/659) were included. Coefficients for the significant predictors of fatal/non-fatal CHD, and CVD were estimated using Cox models. Discrimination and calibration of the United Kingdom Prospective Diabetes Study risk engine (UKPDS-RE), the Framingham Risk Score-Regicor Study (FRS-RS), and the cardiovascular risk model we developed were assessed

Results: Beta coefficients, and standard error (SE) and HRs of risk factors in the best-fitting multivariable 5-year risk model for fatal/non-fatal CHD and CVD are shown in the table. The variable selection procedure identified baseline risk factors for fatal/non-fatal CHD, and CVD. Significant risk factors were age, non-HDL:HDL, and HbA_{1c} for 5-yr risk prediction of CHD and the factors mentioned above, plus SBP and smoking, for 5-yr CVD risk prediction. In the CHD risk prediction equation, high SBP (TA ≥ 140 mmHg) was an independent, significant stratified variable. All of the risk factors that were retained through the variable selection procedure were incorporated into the Basque Country risk engine (BASCORE). Therefore, the risk of fatal/non-fatal CHD increased 24% for each 1% increase in HbA_{1c} or 74% if the non-HDL:HDL was >5. The risk of fatal/non-fatal CVD increased 19% for each 1% increase in HbA_{1c}, 2% for each mmHg of SBP or 19% for each 1 unit of increase in non-HDL:HDL; the risk was reduced by 64% if the patient was not a smoker. The UKPDS-RE and FRS-RS showed inadequate discrimination and calibration for predicting CHD risk. The internal discrimination and calibration of the developed model were acceptable for predicting fatal/non-fatal 2-, and 5-, but not 10-year CHD and CVD risk

Conclusion: This study is the first southern European validated population-derived model for predicting 5-year fatal/non-fatal CHD and CVD risk in newly-diagnosed type 2 diabetic patients

Coronary heart disease			
	Beta coefficients (SE)	Hazard ratio (95% CI)	p value
Age at diagnosis (per 1 year increase)	0.035 (0.010)	1.04 (1.02–1.06)	<0.001
Sex (women vs. men)	0.036 (0.209)	1.04 (0.69–1.56)	0.862
Non-HDL:HDL _{cat} (≥5 vs. <5)*	0.556 (0.223)	1.74 (1.13–2.70)	<0.05
HbA1c at diagnosis (per 1% increase)	0.214 (0.089)	1.24 (1.04–1.48)	<0.05

Cardiovascular disease			
	Beta coefficients (SE)	Hazard ratio (95% CI)	p-value
Age at diagnosis (per 1 year increase)	0.054 (0.008)	1.06 (1.04–1.07)	<0.001
Sex (women vs. men)	-0.211 (0.172)	0.81 (0.58–1.14)	0.220
Non-HDL:HDL (per 1 unit increase)	0.172 (0.072)	1.19 (1.03–1.37)	<0.05
Systolic blood pressure (per mmHg increase)	0.019 (0.006)	1.02 (1.01–1.03)	<0.01
HbA1c at diagnosis (per 1% increase)	0.179 (0.073)	1.19 (1.04–1.38)	<0.05
Tobacco (non-smoker vs. smoker)	-0.448 (0.214)	0.64 (0.42–0.97)	<0.05

*Non-HDL:HDL_{cat}: ratio of non-HDL to HDL cholesterol categorized

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Single and joint effects of obesity and of the metabolic syndrome on cardiovascular event risk

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Background and aims: Obesity is a major risk factor for the metabolic syndrome (MetS), but some obese individuals do not have the MetS while others have the MetS but are non-obese. We prospectively investigated the single and joint effects of obesity and of the MetS on cardiovascular event risk.

Materials and methods: Cardiovascular events were prospectively recorded over 10 years in a large cohort of 1705 patients undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease. Obesity was defined as a BMI ≥30 kg/m²; presence of the MetS was defined according to the current harmonized consensus definition.

Results: From our patients, 827 were non-obese and did not have the MetS, 443 were non-obese but had the MetS, 113 were obese but did not have the MetS, and 322 were obese and had MetS. Cardiovascular event risk was 34.1% in non-obese patients with the MetS. It was significantly higher in this patient group when compared to non-obese subjects without the MetS (25.3%; p<0.001), when compared to obese subjects without the MetS (22.1%; p=0.036), and even when compared to obese subjects with the MetS (25.2%; p=0.006).

Conclusion: We conclude that non-obese patients with the MetS face a particularly unfavourable cardiovascular prognosis.

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Age, insulin requirements, waist circumference and triglycerides predict hypogonadism in type 1 diabetic patients

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Background and aims: The prevalence of hypogonadotropic hypogonadism (HH) in patients with type 2 diabetes mellitus is higher than in the general population and leads to detrimental effects on metabolic control, lipid profile and body composition. Few studies have examined its role in type 1 diabetes mellitus (T1DM). Aims: To characterize patients with T1DM and HH and to evaluate risk factors for this condition.

Materials and methods: Cross-sectional study including T1DM men over 18 years of age with a T1DM duration greater than six months, attended in the outpatient clinic of two hospitals between March–December 2013. A sample of fasting venous blood was taken to measure HbA1c, lipid profile, total testosterone, LH, FSH, SHBG and albumin. History of micro and macrovascular complications, hypertension, dyslipidemia and diabetes duration was gathered. Clinical examination included weight, height, hip and waist circumferences and blood pressure. Metabolic syndrome was diagnosed according to the NCEP-ATP III criteria and the CUN-BAE equation was used to estimate the percentage of body fat. HH was defined as total testosterone less than 8 nmol/L or a calculated free testosterone less than 225 pmol/L, with normal/low levels of LH and FSH. Statistical analysis: Student's t-test was performed to assess differences between two means. For non-normally distributed variables, Mann-Whitney U test was used. Either χ² test or Fisher's exact test was used to examine the degree of association of categorical variables. An experimental model to calculate HH risk was created (HH-Score) using the value of incremental odds for variables predisposing to HH (age, insulin requirements, waist circumference and triglycerides). HH Score = (1.060 x Age) + (1.084 x Waist circumference) + (14.00 x Insulin requirements) + Triglycerides, where age is expressed in years, waist circumference in cm, insulin requirements in IU/kg/d and triglycerides in mg/dL.

Results: 157 patients with a mean age of 44.7 ± 13.1 years and a mean T1DM duration of 19.2 ± 12.7 years were included in the study. HbA1c levels were 7.9 ± 1.3%. Fifteen patients had HH, representing a prevalence of 9.55% (95% CI: 5.0–14.1%).

HH patients presented more frequently hypertension (80.0% vs 47.2%, P < 0.001) and peripheral neuropathy (33.3% vs 12.9%, P < 0.05). No differences in other T1DM complications were detected. An HH-Score > 242.4 showed a 100% sensitivity and a 51.4% specificity for HH diagnosis. The positive and negative predictive values were 17.8% and 100%, respectively.

Conclusion: One in 10 patients with T1DM has HH. A simple formula, including easy and available clinical parameters and triglyceride levels, can predict HH risk; an HH-Score < 242.4 excludes the diagnosis of HH in T1DM patients.

Variables	Hypogonadism (n=15)	No hypogonadism (n=142)	P
Age (years ± SD)	54.6 ± 15.1	43.3 ± 12.2	0.001
BMI (kg/m ² ± SD)	30.9 ± 6.8	25.9 ± 3.4	< 0.0001
Metabolic syndrome (n (%))	12 (80.0)	53 (37.3)	0.001
HbA1c (% ± SD)	8.2 ± 1.4	7.9 ± 1.3	ns
eGDR (mg/kg ⁻¹ ·min ⁻¹ ± SD)	5.4 ± 1.7	6.8 ± 2.1	0.023
Insulin requirements (IU/kg/day ± SD)	0.85 ± 0.2	0.68 ± 0.2	0.010
Hip circumference (cm ± SD)	111.6 ± 15.7	98.9 ± 8.5	< 0.0001
Waist circumference (cm ± SD)	105.8 ± 15.3	93.4 ± 10.1	< 0.0001
Body fat (%) ± SD)	32.3 ± 8.1	25.0 ± 5.3	< 0.0001
Systolic BP (mm Hg ± SD)	146.1 ± 15.0	134.6 ± 15.0	0.005
Diastolic BP (mm Hg ± SD)	78.3 ± 10.3	76.1 ± 9.3	ns
Total Cholesterol (mg/dl ± SD)	175.3 ± 81.9	172.0 ± 29.6	ns
LDLc (mg/dl ± SD)	101.6 ± 61.4	99.3 ± 28.6	ns
HDLc (mg/dl ± SD)	49.7 ± 12.4	55.7 ± 14.7	ns
Triglycerides (mg/dl, median(range))	92.0 (49–700)	74.2 (31–421)	0.058
Neuropathy (n (%))	5 (33.3)	18 (12.7)	0.031
Nephropathy (n (%))	2 (13.3)	20 (14.1)	ns
Retinopathy (n (%))	6 (40.0)	38 (26.8)	ns

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Association between obstructive sleep apneas and complications of type 2 diabetesC. Langrand¹, J. Glerant², F. Gormand³, J. Guerin⁴, P. Moulin¹;¹Endocrinology, Diabetology and Metabolic Diseases, Hôpital Cardiologique Louis Pradel, ²Pneumology, Hôpital Cardiologique Louis Pradel, Bron, ³Pneumology, Centre Hospitalier Lyon Sud, Pierre Benite, ⁴Pneumology, Hôpital de la Croix Rousse, Lyon, France.

Background and aims: Obstructive sleep apneas are highly prevalent in diabetes subjects. Chronic intermittent hypoxia and sleep fragmentation induced by sleep apnea increase sympathetic nervous system activity, oxidative stress and inflammation. These alterations are also involved in the development of diabetes complications, more especially in microvascular complications. The aim of this study was to establish an association between obstructive sleep apnea severity and complications of type 2 diabetes.

Materials and methods: A retrospective observational study was set up in 68 type 2 diabetic patients with obstructive sleep apnea syndrome (OSAS). Respiratory parameters of obstructive sleep apnea and occurrence of diabetes complications were reported between diagnosis of sleep apnea and a mean follow-up of 4 years.

Results: Participants were diabetic subjects with high cardiovascular risk. Most of them presented a severe OSAS (73%). Mean nocturnal oxygen saturation and time spent with oxygen saturation < 90% were significantly impaired in presence of microvascular complications compared to absence of microvascular complications (90% vs. 93% and 29% vs. 7% respectively, $p < 0.005$). Using logistic regression analysis, occurrence of long-term microvascular complications was independently associated with hypoxemia indexes (OR=0.691 [0.530–0.902] $p=0.007$), regardless of adherence to CPAP therapy, age, sex, BMI, HbA1c and diabetes duration. Diabetic nephropathy was predicted both by hypoxemia indexes and diabetes duration. No correlation was found between apnea hypopnea index (AHI) and any diabetes complications. Optimal adherence to CPAP therapy did not improve metabolic parameters or prevent microvascular complications. However, myocardial infarctions were more prevalent in subjects with weak adherence to CPAP therapy (+17% vs. +9%; $p=0.03$).

Conclusion: The independent association between hypoxemia indexes and microvascular complications suggests that chronic intermittent hypoxia induced by sleep apneas could contribute to the progression of microvascular complications in type 2 diabetes. These findings support the need of a specific trial to test the hypothesis of an effect of CPAP therapy in order to prevent microvascular diabetic complications.

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Ectopic fat in the pancreas can predict diabetes complications in non-obese subjects

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Background and aims: Evidence that pancreatic steatosis has a role in obesity, metabolic syndrome and type 2 diabetes mellitus (DM) is emerging. However, data on the influence of pancreatic steatosis on DM complications are lacking.

Materials and methods: We examined 198 patients with type 2 DM. Pancreatic computed tomography (CT) attenuations were assessed using CT imaging. Obesity was defined as BMI ≥ 25 kg/m² according to the Asian-specific BMI cut-offs. We defined pancreatic steatosis as pancreatic attenuations below median levels.

Results: The pancreatic attenuations was significantly correlated with age ($r = -0.302$, $p < 0.001$), visceral fat area ($r = -0.194$, $p = 0.006$) and vascular stiffness ($r = -0.242$, $p = 0.001$). In the non-obese group (BMI < 25 kg/m²), pancreatic steatosis was associated with a higher prevalence of diabetic complications reflecting retinopathy, nephropathy, carotid artery plaque and vascular stiffness. In the non-obese group, patients with pancreatic steatosis, compared with those without, had an odds ratio (OR) of 5.6 (95% confidence intervals [CI] 1.4–22.7) for diabetic retinopathy, 3.5 (95% CI 1.3–9.4) for diabetic nephropathy and 3.1 (95% CI 1.2–8.1) for carotid artery plaque, after adjusting for age, gender and BMI. However, significant associations between pancreatic steatosis and diabetic complications were not found in the obese group.

Conclusion: Pancreatic steatosis is associated with increased diabetic complications in non-obese subjects with type 2 DM. This finding highlights the importance of pancreatic fat deposits associated with the presence of diabetic complications, especially in non-obese subjects.

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Ketosis-prone diabetes is associated with less microvascular complications in sub-Saharan African patientsB. Baz¹, J.-L. Nguewa¹, S.P. Choukem², E. Sobngwi³, M. Ait Djoudi¹, P. Boudou⁴, J.-P. Riveline¹, J.-F. Gautier¹;¹University Center for Diabetes and its Complications, Endocrinology and Diabetology Department, Lariboisiere Hospital, Paris, France, ²Department of Clinical Science, University of Buea, ³National Obesity Center, Endocrinology and Diabetology Department, Central Hospital of Yaounde, Cameroon, ⁴Hormonology Department, Saint Louis Hospital, Paris, France.

Background and aims: Ketosis-prone diabetes (KPD) is a heterogeneous syndrome characterized by ketoacidosis or unprovoked ketosis but do not necessarily have the typical phenotype of autoimmune type 1 diabetes. Data from longitudinally followed cohorts have shown that the clinical features of KPD are “intermediate” between type 1 and type 2 diabetes (T2D). Long-term complications in KPD are not well studied. Our aim was to evaluate the prevalence of long-term microvascular complications in KPD patients compared with T2D patients.

Materials and methods: Data from our KPD cohort register and medical files of T2D patients attending our department were retrospectively analysed and compared. Only patients of sub-Saharan African origin were included.

Results: A total of 80 patients (40 KPD vs 40 T2D) were evaluated. Both groups were matched on sex (female to male ratio 1:4) and diabetes duration (11.5 \pm 4.6 (SD) years). They had similar mean age (55.3 \pm 7.7 vs 56.5 \pm 8.9 years) and mean BMI (28.6 \pm 5 vs 29.4 \pm 5 kg/m²). There was no significant difference between mean HbA1c (8.8 \pm 2.2 vs 8.4 \pm 1.2 $p=0.42$). The number of patients with diabetic retinopathy (18 vs 27 patients) and nephropathy (8 vs 19 patients) were higher in T2D ($p < 0.05$). There was no significant difference between the two groups regarding the diabetic neuropathy prevalence.

Conclusion: Some long-term microvascular diabetes complications are less prevalent in KPD patients compared with T2D patients. The protecting factors and mechanisms remain to be revealed.

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Risk association of triglycerides variability with microvascular complications even in well-controlled type 2 diabetesG. Bardin¹, M. Innocenti¹, C.M. Rotella¹, E. Mannucci², S. Giannini³;¹Obesity Agency, ²Diabetology Agency,³Endocrinology Unit, University of Florence, Italy.

Background and aims: The risk of microvascular complications increases as the mean of glycated haemoglobin (HbA1c) levels increases. Recently, HbA1c variability seems to be an independent risk factor for diabetic microangiopathy. Basic and clinical studies strongly showed that hyperlipidemia accelerates the progression of microangiopathy. In particular, hypertriglyceridemia has an important role in the progression of diabetic kidney disease, while retinopathy (DR) is less clearly associated. Since no data are available, aim of this study was to examine the association between HbA1c and TG variability on the development of DR and nephropathy in type 2 diabetes (T2DM).

Materials and methods: Clinical and anthropometric data, HbA1c and lipid profile were serially recorded in each visit in 450 T2DM clinic outpatients (mean age 68.7 years, with 48.6% females) examined from 2007 to 2014. Patients who had a history of microangiopathy previous the baseline visit were excluded. HbA1c and TG variability, measured as the intrapersonal standard deviation (SD) of serially collected data, were compared in patients who did and did not develop DR or nephropathy. Retinopathy was assessed by dilated funduscopy and diabetic nephropathy was defined based on micro-macroalbuminuria. The association between TG variability and the development of nephropathy was determined by Cox regression analysis adjusted for age, sex, BMI, smoking, duration of diabetes, blood pressure, therapies.

Results: All subjects showed a mean HbA1c 7.2% and a mean duration of disease 13.8 years. Among patients studied, 88.0% were treated only with oral hypoglycemic drugs, 12.0% with insulin, 71.5% with statins and 68.0% assumed renin-angiotensin system antagonists. Over a mean follow-up period of 6.8 years, 9.8% and 27.1% of patients developed retinopathy and nephropa-

thy, respectively. Patients who progressed to retinopathy had higher mean HbA1c ($7.4 \pm 0.8\%$ vs $6.8 \pm 0.7\%$, $p < 0.001$) and SD ($0.63 \pm 0.3\%$ vs $0.50 \pm 0.4\%$, $p < 0.05$) than nonprogressors. Similarly, patients who developed nephropathy had higher mean HbA1c ($7.1 \pm 0.8\%$ vs $6.8 \pm 0.7\%$, $p < 0.05$) and SD ($0.60 \pm 0.4\%$ vs $0.50 \pm 0.4\%$, $p < 0.05$). Considering TG variability, no significant differences were observed between progressors and nonprogressors for retinopathy. Instead, patients who developed nephropathy had higher mean TG (170.2 ± 65.0 mg/dl vs 133.0 ± 47.6 mg/dl, $p < 0.001$) and TG-SD (52.1 ± 30.0 mg/dl vs 35.5 ± 24.2 mg/dl, $p = 0.001$) than nonprogressors. Cox analysis, adjusted for potential risk factors, showed that TG-SD was associated with nephropathy with HR of 2.2 (95% CI 1.2–4.3, $p = 0.014$). Analyzing data from subjects with HbA1c-mean upper or below 7%, in patients with HbA1c $> 7\%$, as expected, the presence of hypertriglyceridemia (TG > 150 mg/dl) increased the prevalence of nephropathy (from 31.6% to 43.0%, $p < 0.001$). Of particular interest, an increased prevalence of nephropathy was also observed in subjects at therapeutic target with HbA1c-mean $< 7\%$ (from 17.4% to 31.0%, $p < 0.001$). Instead, independently of HbA1c levels considered, the TG were not associated to significant differences in the prevalence of retinopathy (from 4.3% to 4.8%, $p = 0.68$).

Conclusion: The TG levels and their variability affect the development of diabetic nephropathy. This effect seems to be additive over HbA1c mean and variability even in patients at target for HbA1c levels.

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Low levels of serum insulin-like growth factor-I increase the risk of osteoporotic fracture and mortality in postmenopausal women with type 2 diabetes mellitus

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Background and aims: Osteoporosis became a social problem in industrialized countries because osteoporotic fractures are associated with low mobility and high mortality. Accumulating evidence has shown that the risk of osteoporotic fractures is increased in type 2 diabetes mellitus. Since the risk is independent of bone mineral density (BMD), the measurement of BMD, which is a gold standard to detect primary osteoporosis, is not useful for screening diabetes-related bone fragility. Therefore, it is an important issue to find evaluation tools to assess the risk of future osteoporotic fracture instead of BMD measurement in type 2 diabetes. Insulin-like growth factor-I (IGF-I) is well-known to have anabolic effects on bone. In addition, serum IGF-I levels were reported to be associated with the presence of vertebral fracture. We previously showed that serum IGF-I levels was inversely associated with the prevalence and severity of vertebral fractures independent of BMD. We thus hypothesized that serum IGF-I was involved in the etiology of diabetes-related bone fragility and its serum levels might be a clinically useful marker for assessing future fracture risk in type 2 diabetes mellitus. In this study, we examined the association between serum IGF-I levels and the incidence of fracture as well as mortality in postmenopausal women with type 2 diabetes mellitus.

Materials and methods: In this study, we recruited 197 postmenopausal women with type 2 diabetes mellitus whose serum IGF-I levels and BMD at lumbar spine (L-BMD) were previously measured from 1993 to 2009 at Shimane university hospital. Medical doctors interviewed the history of the subjects with special attention to osteoporotic fractures in 2013. Student's *t* tests and multiple regression analyses were performed to examine the association of the incidence of osteoporotic fracture and death after IGF-I measurement with serum IGF-I levels.

Results: Of 197 subjects, 27 patients died, and 24 patients suffered from new osteoporotic fracture. Serum IGF-I levels were marginally lower in patients with osteoporotic non-vertebral fractures than in those without them (118 ± 38 ng/mL vs 140 ± 53 ng/mL, $p = 0.054$). Moreover, serum IGF-I levels were significantly lower in dead patients than in survivors (94 ± 50 ng/mL vs 137 ± 52 ng/mL, $p < 0.001$). Multiple logistic regression analysis adjusted for age, duration of diabetes, body mass index, HbA1c, serum creatinine, L-BMD showed that serum IGF-I levels were significantly and inversely associated with the incidence of osteoporotic non-vertebral fractures [odds ratio (OR) = 0.49, 95%CI 0.24–0.99, $p = 0.049$] as well as with all-cause death (OR = 0.45, 95%CI 0.21–0.95, $p = 0.035$).

Conclusion: The present study showed for the first time that serum IGF-I levels were associated with the incidence of osteoporotic non-vertebral fractures and death in postmenopausal women with type 2 diabetes, suggesting

that serum IGF-I could be clinically useful for assessing the risk of future osteoporotic fractures as well as mortality in postmenopausal women with type 2 diabetes mellitus.

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Metabolic and vascular characteristics of insulin resistance in Korean patients with type 2 diabetes

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Background and aims: Insulin resistance (IR) is a paramount concern of type 2 diabetes and its associated disorders. To investigate the association of IR with metabolic and vascular disorders related to diabetes, we analyzed 7,109 Korean type 2 diabetic patients according to IR.

Materials and methods: A total 7,109 patients with type 2 diabetes were recruited through our Diabetes Center from January 2003 to June 2009. Insulin sensitivity was measured by a rate constant for plasma glucose disappearance (Kitt, %/min) using short insulin tolerance test. Subgroup analyses were performed according to the tertiles of Kitt. Carotid atherosclerosis was defined as presence of isolated focal plaque or mean intima-media thickness (IMT) more than 1.0 mm.

Results: Mean age was 58.1 ± 10.1 years old, and 71.5% of diabetic patients had IR. Patients with the lowest tertile of Kitt (IR group) showed higher levels of metabolic parameters such as body mass index (BMI), visceral fat thickness, blood pressure, fasting blood glucose, HbA1c, total cholesterol, triglyceride and LDL cholesterol but lower HDL cholesterol compared to the patients with the highest tertile of Kitt (insulin-sensitive group). Mean and maximal IMT of carotid arteries was significantly higher in the patients with the lowest tertile of Kitt. As the value of Kitt was lower, the prevalence of metabolic and vascular disorders related to diabetes increased. In multiple regression analysis, IR was an independent risk factor for the metabolic and vascular disorders related to diabetes after adjusting for age, sex, duration of diabetes, BMI and HbA1c. Odds ratios for metabolic syndrome and carotid atherosclerosis in patients with the lowest tertile of Kitt were 3.108 (95% CI, 2.721–3.549) and 1.232 (95% CI, 1.069–1.418), respectively.

Conclusion: IR is not only prevalent but also an important predictor for metabolic and vascular disorders in Korean patients with type 2 diabetes. Beyond reducing the HbA1c, management focusing on IR such as life-style modification or using insulin-sensitizing agents should be evidently considered in patients with type 2 diabetes.

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Multi-morbidity in type 2 diabetes: systems biology analysis based on 196,627 patients from a real-life multicentre analysis

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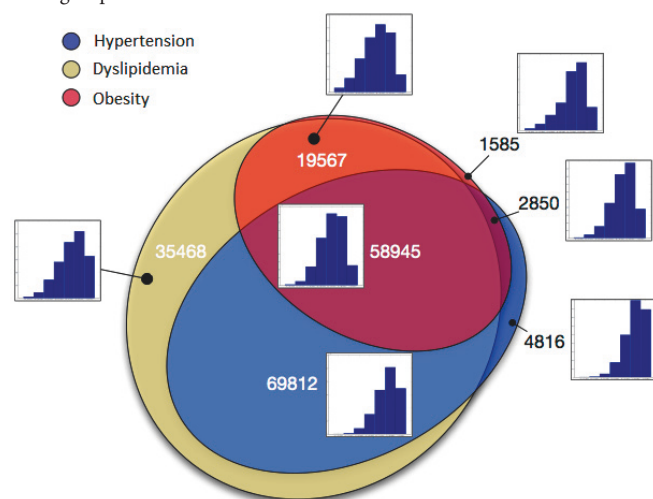
Background and aims: Patients with type 2 diabetes (T2D) often have various chronic comorbid diseases. The aim was to investigate electronic medical record data to examine the incidence of multi-morbidity as well as the co-occurrence of T2D with the most prevalent diagnoses and to attempt to stratify metabolic syndrome.

Materials and methods: A cohort of 196,627 T2D patients from the German/Austrian Prospective Diabetes Follow-up Registry (DPV) was analyzed. Data included gender, age, duration of diabetes and the additional diagnosis of the 20 most prevalent diseases. Proportional Venn diagrams were used to visualize patterns in the data. Subgroup analyses were compared with results from classical logistic regression models (SAS 9.4).

Results: The maximum number of comorbidities was 13. 95% of the patients had less than 6 diseases, 1.4% of the patients had no comorbidity. As expected, the most prevalent diseases were dyslipidemia (93.5%), hypertension (69.4%) and obesity (42.2%), matching the traditional components of metabolic syndrome. The next most prevalent comorbidities were polyneuropathy

(12.9%) and retinopathy (10.1%). Female and male prevalence of all diseases except stroke, fatty liver and retinopathy differed significantly (all $p < 0.01$). Prevalence of obesity and fatty liver decreased with increasing age, all other disease distributions increased or remained constant. Proportional Venn diagrams revealed that obesity and hypertension were mostly combined with dyslipidemia (94.4% and 94.7%, respectively). However, the proportions differed with age: young patients were more obese, older patients were more hypertensive. The proportion of both obesity and hypertension increased with longer duration of diabetes. In contrast, duration of diabetes appeared to have little effect on the proportion of dyslipidemia. It is remarkable that a considerable fraction of T2D patients was not obese, and that some patients seemed to be resistant to diabetes-induced hypertension. Based on Venn diagrams metabolic syndrome was stratified into four main groups. Prevalence of additional comorbidities and their risk factors was different in these groups. For retinopathy, logistic regression models revealed duration of diabetes as risk factor in all 4 groups, whereas age was not significant in the 'hypertension and dyslipidemia' group. Both visualization and regression approach indicated interactions between age and duration of diabetes.

Conclusion: We found a high prevalence of multi-morbidity in T2D patients, with dyslipidemia being almost omnipresent in all age groups. The concept of "metabolic syndrome" can be clearly visualized and stratified into 4 main types. The emergence of the additional comorbidities appears different in these groups.



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PS 110 Biomarkers and complications I

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Alanine aminotransferase and mortality in patients with type 2 diabetes mellitus (ZODIAC-43)

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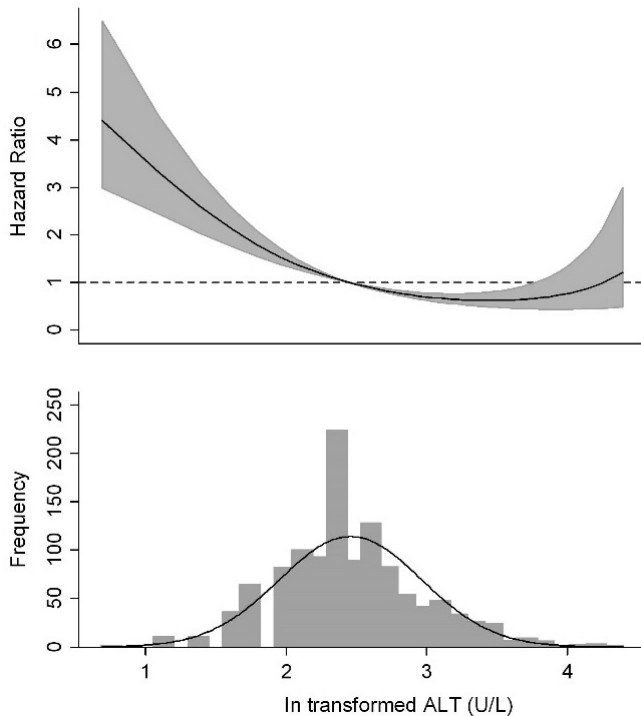
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Background and aims: Combined data suggests the existence of a bimodal association of alanine aminotransferase (ALT) with mortality in the general population. Little is known about ALT as a predictor of mortality in patients with type 2 diabetes mellitus. We aimed to investigate the association of ALT with all-cause, cardiovascular, and non-cardiovascular mortality in patients with type 2 diabetes mellitus.

Materials and methods: A prospective cohort study was performed in patients with type 2 diabetes mellitus, treated in primary care, participating in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study. Cox regression analyses were performed to determine associations of log-transformed baseline ALT with all-cause, cardiovascular, and non-cardiovascular mortality.

Results: In 1,187 patients with type 2 diabetes mellitus, mean age was 67 ± 12 years and 46% was male. Median (interquartile range) ALT levels were 11 (8–16) U/L. ALT levels were above the upper limit of normal (45 U/L) in sixteen (1.3%) patients. During median follow-up for 5.5 (3.1–10.1) years, 354 (30%) patients died, with 149 (42%) attributable to cardiovascular causes. ALT was inversely associated with all-cause mortality (HR 0.75; 95%CI 0.61–0.92; $P=0.006$). Furthermore, ALT was not associated with cardiovascular mortality (HR 0.94; 95% CI 0.68–1.29; $P=0.68$), but particularly with non-cardiovascular mortality (HR 0.65; 95%CI 0.50–0.86; $P=0.002$). These associations were independent of potential confounders including age, sex, body mass index, cholesterol/HDL ratio, smoking, systolic blood pressure, diabetes duration, serum creatinine, HbA1c, cardiovascular history, treatment with ACE inhibitors, and albumin to creatinine ratio. In addition, there appeared to be a bimodal association of ALT and all-cause mortality ($P=0.013$, Figure 1), with a similar bimodal trend for non-cardiovascular mortality.

Conclusion: In conclusion, our findings suggest that low levels of ALT are associated with a high risk of all-cause mortality, in particular of non-cardiovascular mortality, in patients with type 2 diabetes mellitus with ALT levels largely within the normal range.



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Serum PEDF levels in type 2 diabetes: correlations, predictive power for vascular events and increases by fenofibrate in the FIELD Study

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Background and aims: Pigment Epithelium Derived Factor (PEDF) is an endogenous glycoprotein with potent anti-angiogenic, anti-inflammatory and anti-oxidant effects and links with lipoprotein metabolism. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study of Type 2 diabetes subjects we aimed to determine if baseline serum PEDF levels (i) are correlated with traditional and novel vascular risk factors; (ii) are associated with on-trial occurrence of microvascular and cardiovascular complications over 5-year follow-up; and (c) are changed by allocation to (up to one year of) 200 mg daily comiconised fenofibrate.

Materials and methods: PEDF levels were quantified by ELISA (Merck Milipore, USA) with CVs <10% in sera from (n=8898) FIELD Study participants at baseline and after a 16 week run-in period, including 6 weeks of daily oral 200 mg fenofibrate and in 1832 FIELD subjects after one year of allocation to fenofibrate or placebo. Risk factors evaluated included: BMI, lipid levels, HbA1c, renal dysfunction, insulin and HOMA-IR, leptin, inflammation (including sVCAM-1, sICAM, se-Selectin, IL-6, CRP and fibrinogen) and oxidative stress (OxLDL and myeloperoxidase (MPO)). Tertiles of PEDF at baseline were related to composite CVD (CVD death, MI, stroke or coronary or carotid revascularization) and microvascular (retinal laser, albuminuria, neuropathy or microvascular amputation) end-points with and without adjustment for covariates. Covariates were age, sex, diabetes duration, HbA1c, systolic BP, fasting triglycerides, LDL-C and HDL-C, smoking, and baseline glucose control treatment and (for CVD only) urinary albumin creatinine ratio. Statistical significance was taken at $p < 0.05$.

Results: Baseline PEDF levels correlated (weakly) (Spearman correlation r values 0.05–0.30, all $p < 0.0001$) with: baseline HbA1c, blood pressure, triglycerides, LDL-C and HDL-C (inverse), BMI and leptin, renal dysfunction (elevated plasma creatinine, cystatin C, lower calculated GFR and increased albuminuria), insulin levels and HOMA-IR, and with measures of inflamma-

tion (cell adhesion molecules, IL-6, CRP, fibrinogen) and MPO ($p = 0.002$). On unadjusted analyses baseline PEDF tertiles were predictors of on-trial CVD ($n = 1199$, HR up to 1.33, $p < 0.001$) and microvascular events ($n = 3465$, HR up to 1.47, $p < 0.001$). With adjustment for all covariates (above) the relationship with subsequent CVD events was not statistically significant, whilst that with microvascular events was. In the active run-in period including 6 weeks fenofibrate, PEDF levels increased 18 (95% CI 17–19) % from baseline levels. Over one year PEDF levels increased by 16.6 (14.1–19.0) % in the group allocated fenofibrate ($n = 917$; $p < 0.0001$) vs. a 3.4 (1.6–5.9) % increase in the placebo allocated group ($n = 915$; $p < 0.006$).

Conclusion: In Type 2 diabetes patients in the FIELD Study serum PEDF levels correlated significantly, albeit weakly, with traditional and novel vascular risk factors, including renal dysfunction, adiposity, inflammation and oxidative stress. Baseline PEDF levels predict on-trial microvascular and CVD and events. PEDF levels are significantly increased by fenofibrate. We suggest that some of fenofibrate's clinical effects may be via effects on PEDF and PEDF may be a therapeutic target.

Clinical Trial Registration Number: 64783481

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Association of the alternative pathway of complement activation with incident cardiovascular diseases: the CODAM study

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Background and aims: The complement system has been implicated in the development of both type 2 diabetes (DM2) and cardiovascular diseases. Complement can be activated spontaneously via the alternative complement pathway, which functions also as potent amplifier of other routes of complement activation. We measured plasma properdin and factor D - a positive regulator and a protease, respectively, of the alternative pathway, and factor Bb - an alternative pathway activation marker, and investigated their associations with incident cardiovascular diseases in a diabetes-prone population.

Materials and methods: We conducted prospective analyses in the CODAM study (Cohort on Diabetes and Atherosclerosis Maastricht, baseline examination 1999–2001; follow-up examination 2006–2009). Cardiovascular events (CVE) included myocardial infarction (self-reported or identified with ECG-examinations), self-reported stroke, self-reported cardiac angioplasty and/or cardiac bypass. Cardiovascular disease (CVD) additionally included signs of ischaemia on an ECG and/or ankle-brachial index <0.9. We included 371 individuals who were free of CVE at baseline (59% men, age 58.2 ± 6.9 years, 21% DM2). Participants with CVD at baseline ($n = 53$) were additionally excluded in analyses of incident CVD. Associations between properdin, factor D and Bb at baseline and incident CVE and CVD were studied with logistic regression analyses adjusted for baseline age, sex, impaired glucose metabolism, DM2, blood pressure, BMI, smoking, physical activity, renal function, and use of medication. We examined effect modification by prevalent DM2.

Results: After a mean follow-up time of 7.1 ± 0.1 years, there were 48 incident CVE and 65 incident cases of CVD. Properdin was independently associated with CVE (per 1SD increase, OR=1.50, [95%CI: 1.10–2.06], $P = 0.011$) but not CVD (OR=1.09, [0.81–1.46], $P = 0.58$). Bb tended to be associated with CVD (per 1SD, OR=1.30, [0.98–1.71], $P = 0.065$) but not with CVE (OR=1.06, [0.76–1.46], $P = 0.75$). Factor D was in the whole population not associated with CVD (per 1SD, OR=1.32, [0.92–1.88], $P = 0.14$) or CVE (OR=1.17, [0.80–1.72], $P = 0.43$), but effect modification by DM2 was observed in the association with CVD ($P_{\text{factor D} \times \text{DM2}} = 0.049$). In stratified analyses, factor D was associated with CVD (OR=4.42, [1.29–15.16], $P = 0.018$) only in participants with baseline DM2 ($N = 61$, 19 cases).

Conclusion: In CODAM, higher levels of properdin were independently associated with CVE and higher levels of Bb tended to be associated with CVD. In participants with DM2, factor D was independently associated with CVD. This suggests that the alternative complement pathway may play a role in the development of cardiovascular diseases both in individuals with and without DM2.

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Circulating resistin levels and mortality risk

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Background and aims: Studies concerning the association between circulating resistin levels and mortality yielded mixed **Results:** some studies reported significant associations, while many others showed negative results. To address this question, we analyzed data from European subjects with type 2 diabetes and evidence of coronary artery disease (CAD), a prospective cohort followed over time for cardiovascular and all-cause mortality. To assess the consistency of the association between resistin and mortality risk, we also performed a meta-analysis of prospective studies.

Materials and methods: We investigated: i) the Gargano Heart Study (GHS) prospective design (n=359 diabetic patients; 81 and 58 incident cases of all-cause and cardiovascular (CV) mortality, respectively); ii) meta-analyzed in a dose-risk fashion our present data from GHS study and all studies from MEDLINE and EMBASE until February 2014 reporting adjusted hazard ratios (HR) of circulating resistin for all-cause or CV mortality.

Results: In GHS, the adjusted HRs per 10 ng/ml resistin increment were: 1.45 (95% CI: 1.10-1.91) and 1.52 (95% CI: 1.09-2.12) for all-cause and CV mortalities, respectively. The dose-risk meta-analyses included 7 studies (n=3,699, 997 events) for all-cause mortality and 6 studies (n=4,187, 412 events), for CV mortality. Pooled HRs per 10 ng/ml resistin increment were 1.33 (95% CI: 1.03- 1.72, p=0.028, Q-test p for heterogeneity<0.001) and 1.16 (95% CI: 0.95-1.43, p=0.147, Q-test p for heterogeneity=0.039) for all-cause and CV mortality, respectively. Due to the presence of between-studies heterogeneity, meta-regression analyses were performed. For all-cause mortality, study mean age (HR=1.08, 95%CI=1.01-1.14, p=0.017) explained 68.8% of heterogeneity resulting in a pooled HR per 10 ng/ml resistin increment equal to 1.33 (95% CI: 1.12-1.57, p=0.001). For CV mortality, study mean BMI (HR=1.07, 95%CI=1.02-1.11, p=0.004) explained the whole heterogeneity resulting in a pooled HR per 10 ng/ml resistin increment equal to 1.11 (95% CI: 1.02-1.22, p=0.016).

Conclusion: Our results provide strong evidence for an association between higher circulating resistin levels and increased mortality risk.

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Prognostic value of plasma NT-proBNP levels in asymptomatic diabetic patients

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Background and aims: Elevated plasma N-terminal (NT)-proBNP levels predict adverse cardiovascular events in the general population and in coronary patients. We previously showed that NT-proBNP is also a marker of silent coronary artery stenoses (CS) in diabetic patients without cardiac history or symptom. The aim of this study was to determine the prognostic value of plasma NT-proBNP levels independent from silent coronary disease and left ventricle hypertrophy (LVH).

Materials and methods: We included 323 diabetic patients (17 type 1, 306 type 2; 185 men, 138 women) without history or sign of cardiac disease but with at least one additional risk factor including nephropathy in 41% of the patients. Silent myocardial ischaemia (SMI) was assessed using stress myocardial scintigraphy and CS using coronary angiography in those with SMI. Left ventricular mass was evaluated reliably by echocardiography (ASE convention) in 282 of them. LVH was defined as LV mass ≥ 106 (women) or 110 g/m² (men). Plasma NT-proBNP was measured.

Results: SMI was detected in 108 patients, 39 of whom had CS, and LVH in 92 patients. The highest tertiles of NT-proBNP were associated with a higher prevalence of hypertension (p<0.0001), nephropathy (0.01), peripheral vascular disease (0.03), LVH (19.4, 35.1, 43.2% ; p<0.001) and CS (5.6, 10.2, 20.4% ; p<0.001). At follow-up (4.6 \pm 2.6 years), 29 patients had a cardiovascular event (10 acute coronary syndrome, 4 cardiac death, 5 heart failure, 4 stroke, 1 amputation, 5 revascularisations). CS and the highest NT-proBNP

tertile (≥ 38 pg/ml) were significant predictors of events (p=0.01 and 0.002, respectively). In multivariate analysis including SMI or CS the highest tertile of NT-proBNP predicted the events independently from SMI (OR 2.9 [1.4-6.0], p<0.005) and CS (OR 2.4 [1.1-5.2], p=0.02). In another model including also LVH the highest tertile of NT-proBNP was still an independent predictor (p<0.0001 and p=0.003, respectively).

Conclusion: This study suggests that in asymptomatic diabetic patients NT-proBNP is a risk marker for cardio-vascular events independent from coronary status and LVH.

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Comparison of predictive capabilities of MR-proANP and NT-proBNP for mortality (ZODIAC-42)

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Background and aims: The midregional fragment of pro-A-type natriuretic peptide (MR-proANP) has been suggested as an alternative for N-terminal pro-B-type natriuretic peptide (NT-proBNP), an established biomarker in heart failure. A previous head-to-head comparison in the general population between both peptides showed that MR-proANP was as efficient as NT-proBNP in predicting all-cause mortality and cardiovascular events. However, the association of MR-proANP with cardiovascular mortality was not independent from confounders. We aimed to compare the predictive capability of MR-proANP and NT-proBNP for all-cause and cardiovascular mortality in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: Patients were selected from two cohorts (1998 and 2001) of the prospective observational ZODIAC study (n=1688). For this study, 541 patients were excluded because plasma MR-proANP and/or plasma NT-proBNP were not measured. A Cox proportional hazard model was used to investigate the relationship between the two natriuretic peptides and (cardiovascular) mortality. We used three models: a crude model, an age- and gender-adjusted model, and a model in which we additionally adjusted for smoking (yes or no), BMI, duration of diabetes, HbA1c, serum creatinine level, macrovascular complications (yes or no), albuminuria (yes or no) and total cholesterol-HDL ratio. Harrell's C statistic was used to compare between models with MR-proANP and those with NT-proBNP.

Results: After a median follow-up period of 11 years, 552 (48%) patients died of which 239 patients (43%) died from cardiovascular causes. The median (interquartile range) values for plasma concentrations of MR-proANP and NT-proBNP were 74 pmol/L (48-120 pmol/L) and 11 pmol/L (4-27 pmol/L), respectively. The results of the Cox regression analyses are presented in table 1. Increased plasma concentrations of both natriuretic peptides are independently associated with all-cause and cardiovascular mortality. The Harrell's C values of the models for all-cause mortality are similar for both peptides. For cardiovascular mortality, the Harrell's C values of the models with NT-proBNP are higher compared to the models with MR-proANP.

Conclusion: This study is the first study showing that MR-proANP is related to cardiovascular mortality after adjustment for cardiovascular risk factors. The predictive performance of both peptides is similar for all-cause mortality. Regarding cardiovascular mortality, NT-proBNP seems to perform slightly better.

Table 1. Results of the Cox regression analyses of the logarithmically transformed MR-proANP and NT-proBNP data, and the comparison of their capability for predicting all-cause and cardiovascular disease (CVD) mortality.

	MR-proANP		NT-proBNP	
	HR (95%CI)	Harrell C (95%CI)	HR (95%CI)	Harrell C (95%CI)
All-cause mortality				
- Unadjusted	3.85 (3.35-4.42)	0.73 (0.71-0.75)	1.85 (1.74-1.97)	0.74 (0.72-0.76)
- Model 1	2.15 (1.83-2.52)	0.78 (0.76-0.80)	1.48 (1.38-1.59)	0.78 (0.77-0.80)
- Model 2	2.01 (1.68-2.40)	0.80 (0.78-0.82)	1.40 (1.29-1.52)	0.80 (0.78-0.82)
Cardiovascular mortality				
- Unadjusted	5.02 (4.06-6.20)	0.76 (0.73-0.79)	2.12 (1.93-2.33)	0.78 (0.75-0.81)
- Model 1	3.18 (2.49-4.06)	0.79 (0.76-0.82)	1.79 (1.60-1.99)	0.81 (0.78-0.83)
- Model 2	2.51 (1.92-3.29)	0.83 (0.81-0.85)	1.57 (1.40-1.77)	0.84 (0.81-0.86)

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ProBNP strongly predicts future macrovascular events in angiographed coronary patients as well as in those without the metabolic syndrome

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Background and aims: Pro-B-type natriuretic peptide (proBNP) is a prognostic biomarker for patients with congestive heart failure as well as in other patient populations. The power of proBNP to predict cardiovascular endpoints in patients with the metabolic syndrome (MetS) is unclear and is addressed in the present study.

Materials and methods: We measured serum proBNP in 722 patients undergoing coronary angiography for the evaluation of stable coronary artery disease (CAD). Significant CAD was diagnosed in the presence of coronary stenoses with lumen narrowing of $\geq 50\%$. Prospectively, we recorded vascular events over 3.2 ± 1.2 years.

Results: ProBNP was significantly higher in patients with ($n=386$) than in subjects without significant CAD at baseline (711 ± 1287 vs. 663 ± 1565 pg/ml; $p=0.001$). Prospectively, we recorded 121 cardiovascular events. The incidence of vascular events significantly increased over tertiles of proBNP in patients with the MetS (10.7%, 18.5%, and 28.8% respectively; $p=0.004$) as well as in those without the MetS (10.4%, 11.5%, and 22.0%, respectively; $p=0.011$). Similarly, serum proBNP significantly predicted the incidence of major cardiovascular events after adjustment for age, gender, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol and the eGFR both in subjects with the MetS (standardized adjusted HR 1.48 [1.21–1.80]; $p < 0.001$) and in those without the MetS (HR 1.21 [1.04–1.40]; $p=0.011$). These results were not attenuated after further adjustment for the angiographically determined baseline CAD state (HRs 1.50 [1.23–1.83]; $p < 0.001$ and 1.26 [1.09–1.47]; $p=0.003$ in subjects with the MetS and in those without the MetS, respectively).

Conclusion: Serum proBNP predicts cardiovascular events independently of established cardiovascular risk factors and of the baseline coronary artery state both in patients with and in subjects without the MetS.

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Type 2 diabetes mellitus in patients with terminal stage heart failure

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Background and aims: Type 2 diabetes mellitus is a major independent risk factor for the development of heart failure. Prevalence of type 2 diabetes in patients with heart failure is higher than in general population. The cardiovascular disease mortality is higher in patient with diabetes and heart failure concurrently than in nondiabetics with failing heart. Based on these knowledge, our interest was to compare diabetic and nondiabetic patients in terminal stage heart failure and assess possible differences in routinely measured and recorded clinical parameters. On molecular level, we suspected deterioration of gene expression of cardiac myosin heavy chain isoforms and regulatory microRNAs originating from these isoforms. Possible shift in expression of these main components of cardiac contractile apparatus may be the underlying source of increased cardiovascular mortality in diabetic patients.

Materials and methods: Cohort consisted of 42 patients (38 male, 4 female) with terminal stage heart failure. Inclusion criteria were: heart failure classified NYHA III – IV, patients were indicated for orthotopic heart transplantation, the recorded underlying cause of heart failure was dilated cardiomyopathy or coronary artery disease (two most frequent causes). 16 of 42 patients was diagnosed type 2 diabetes previous to the heart transplantation. We tested the differences in clinical parameter: age, blood pressure, body mass index, data from right heart catheterization, echocardiography, electrocardiography, blood biochemistry, anamnesis of co-morbidities, interventional procedures and complete pharmacotherapy. Expression of cardiac myosin heavy chain isoforms (MYH6, MYH7, MYH7B) and microRNAs originating from their introns (miR-208a, miR-208b, miR-499 respectively) were analyzed in samples from left ventricular free wall of explanted hearts, using RT-qPCR.

Results: The most prominent difference between diabetic and nondiabetic patients was the expected difference in blood glucose ($** p = 0.002$; 8.7 ± 4.0 vs. 5.8 ± 1.3 mmol/l respectively; mean \pm SD). The only other detected differences were the values of uric acid ($** p = 0.006$; 610.1 ± 207.3 vs. 450.8 ± 136.1 μ mol/l) and urea ($*** p < 0.001$; 11.1 ± 4.1 vs. 7.1 ± 2.8) contributing to altered metabolic state or perhaps resulting from modulated renal function. All other clinical parameter assessing heart function or blood biochemistry are indistinguishable between the two groups of patients. The same applies also for the indifference in gene expression of myosin heavy chain isoforms MYH6, MYH7, MYH7B and their innate microRNAs (miR-208a, -208b, -499).

Conclusion: In terminal stage of failing heart there is no clinical parameter, that would indicate even more deteriorate heart function in diabetic patients then it is all ready damaged in nondiabetic counterparts. The same is true also for gene expression of main motor proteins, cardiac myosin heavy chain isoforms and their innate regulatory microRNAs. Our results show that currently there is no indicator for deterioration of cardiac function in patient with diabetes in the terminal stage of heart failure.

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Head to head comparison of inflammatory and metabolic biomarkers for the long-term prediction of new onset left ventricular diastolic dysfunction in type 2 diabetes patients

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Background and aims: Left ventricular diastolic dysfunction in patients with diabetes is commonly used as an imaging finding of diabetic cardiomyopathy in the absence of other etiological factors. With this 48month prospective study we investigated the predictive value of the classic and novel biomarkers on the new onset left ventricular diastolic dysfunction in type 2 diabetes patients.

Materials and methods: We recruited 48 type 2 diabetes patients (45.83% males) of mean age 55.45 ± 10.13 years with mean duration of diabetes 2.81 ± 2.50 years with normal both systolic and diastolic cardiac function. Exclusion criteria were systolic or diastolic cardiac impairment, coronary heart disease, arrhythmias or valvular diseases and history of other chronic inflammatory states (e.g. COPD or autoimmune diseases). All recruits were examined annually for 4 years for: BMI, FPG, HbA1c, eGFR, lipidemic profile, aminotransferases, uric acid, BNP, hs-CRP, fibrinogen and the newer inflammatory biomarker soluble ST2. Complete echocardiograms at same time points revealed those that switched from normal to impaired diastolic function, according to the latest A.H.A./E.S.C. criteria.

Results: Left ventricular diastolic dysfunction (impaired relaxation pattern) was present at the 54.17% of our sample by the end of the study (switchers). The remaining 45.83% preserved their normal cardiac function (non switchers) throughout the 48month observation period. Statistical significant differences between switchers - non switchers were found only for: BMI (31.0 ± 4.7 vs 27.5 ± 5.0 , $p=0.024$), FPG (148 ± 35 vs 125 ± 36 , $p=0.011$), HDL-C (42 ± 10 vs 52 ± 19 , $p=0.041$), triglycerides (139 ± 35 vs 106 ± 64 , $p=0.034$), uric acid (6.3 ± 1.4 vs 5.0 ± 1.4 , $p=0.008$) and hs-CRP (7.9 ± 7.1 vs 2.8 ± 3.2 , $p=0.011$). No correlation was found with the rest of the study parameters. Mean serum soluble ST2 for switchers was 11.5 ± 3.4 ng/mL while for non switchers 11.0 ± 2.4 ng/mL ($p=0.58$). Multivariate regression analysis revealed hs-CRP and triglycerides as the only study parameters that can predict the new onset of left ventricular diastolic dysfunction in our type 2 diabetes population.

Conclusion: High sensitivity CRP along with triglycerides act as independent predictive biomarkers for the instatement of new onset left ventricular diastolic dysfunction in type 2 diabetes patients, pattern not proven for the soluble ST2, fibrinogen, BNP and other metabolic parameters.

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Protective effect of telomere length in patients with type 2 diabetes mellitus

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Background and aims: Type 2 diabetes mellitus (T2DM) is the primary risk marker influencing risk of cardiovascular disease. It is known that telomere length (TL) shortening is a marker of cell aging and associated with increased arterial stiffness (AS) in patients with T2DM, despite the patient's age, leading to the vascular aging. The aim of the study was to compare the vascular and cellular aging in patients with and without T2DM.

Materials and methods: TL was assessed by quantitative polymerase chain reaction (PCR) in 98 patients with T2DM (mean age 61±2,6 years) and in 101 healthy patients in mean age of 51±1,8 years. Intima media thickness (IMT) and plaque presence (PP) were determined by ultrasonography in both left and right carotid arteries. AS was appreciated by aortic pulse wave velocity (PWV) measuring by SphygmoCor (AtCor Medical).

Results: The median of telomere length (TLL) was 9,75. «Short» telomeres were considered if the telomere length was 9.75. All patients were divided into 4 groups by TL - «long» (T2DM+ (n=57) and T2DM- (n=49)) and «short» telomeres ((T2DM+ (n=41) and T2DM- (n=52)). Comparison of vascular aging parameters in patients with long telomeres showed that the state of vessels in T2DM were as similar as in healthy people - they had a lower arterial stiffness and other signs of vascular ageing : PWV 10,58±0,1 (T2DM+) vs 10,5±0,5 m/s (T2DM-), p=0,913; IMT 0,904±0,09 (T2DM+) vs 0,77±0,03 mm (T2DM-), p=0,1227; PP 0,886±0,4 (T2DM+) vs 0,782±0,2 (T2DM-), p=0,979. In contrast in patients with «short» TL and DM PWV was significantly higher than in non-diabetic people (15,08±1,3 vs 10,7±0,5 m/s, p=0,0151), there were greater number of plaques (1.125±0,29 vs 0,789±0,22, p=,04); IMT was 0,87±0,1 (T2DM+) vs 0,78±0,1 mm (T2DM-), p=0,1814.

Conclusion: In patients with short TL and T2DM the severity of vascular disorders is higher than in healthy people. In contrast, in patients with long TL with T2DM there are no significant differences in the vascular structure as compared with healthy individuals. Despite the presence of T2DM signs of vascular aging are minimal in patients with long TL. Short TL may be regarded as non-hemodynamic components of rapid vascular ageing in patients with T2DM.

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Sleep apnoea syndrome and type 1 diabetes: impact on macroangiopathies

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Background and aims: Sleep Apnoea Syndrome (SAS) is an independent cardiovascular morbi-mortality factor that is undoubtedly enhanced when associated with T1D. The aim of this study is to assess prevalence and profile of macroangiopathies in this situation (T1D + SAS), more precisely blood pressure and potential conduction defects (QT interval).

Materials and methods: SAS was found in 28 patients (20.7%) from a 135 T1D cohort consecutively enrolled with Apnoea Hypopnoea Index (AHI) ≥15/h confirmed with polysomnography. Among them, respectively 50% and 50% suffer from severe (AHI≥30/h) or moderate (AHI≥ 15 ≤29/h) SAS.

Results: Patients' characteristics at enrolment were: age 64 ± 12.9 years, diabetes duration 27.1 ± 13.7 years, 75% of males, HbA1c 8.0 ± 1.0% (39.3% with HbA1c≤7.5%), weight: 75.2 ± 18.8kg, BMI : 26.5 ± 5.8 kg/m² (50% lower than 25kg/m², 21.4% between 25 and 26.9kg/m² et 28.6% higher than 27 kg/m²). Among them, 21 patients (75% of SAS T1D and 15.5% of all T1D) show at least one macroangiopathic damage (MA+): 8 coronaritis, 15 carotid damages, 13 lower limb arteriopathy). MA+ patients were compared to MA- patients on HbA1c (8.0 vs 8.0%), BMI (27.2 vs 24.7 kg/m²), creatinemia (10.4 vs 8.2mg/l), total cholesterol (1.8 vs 2.1g/l), triglyceride level (1.4 vs 0.6g/l), neuropathy

frequency (81 vs 57.1%), nicotine addiction frequency (14.3 vs 0%), Hypoxia (AHI : 30.4 vs 23.9/h ; 57.1 vs 28.6% of severe SAS and 42.9 vs 71.4% of moderate SAS). We have also studied blood pressure (SBP : 126.5 ± 15.3 vs 126.4 ± 15.5mmHg/DBP : 75.6 ± 7.7 vs 73.6 ± 10.3mmHg), Mean Arterial Pressure (MAP: 93.9 ± 9.2 vs 97.0 ± 3.0mmHg) and Pulse Pressure (PP: 50.9 ± 17.2 vs 55.0 ± 13.8mmHg). The electrocardiogram of MA+ patients shows mean QT and QTC intervals of 401.2 ± 42.5 et 414.9 ± 22.4 mm/s while the one of a 22 patients control group (C) with no SAS and no macroangiopathy shows mean QT and QTC intervals of 382.9 ± 22.5 et 398.8 ± 12.7 mm/s. There is a significant difference in QTC intervals (p = 0.012). No heart rhythm disorder is observed. SBP, DBP, MAP and PP have been compared too (SBP: 126.5 ± 15.3 vs 124.7 ± 11.4 mmHg - NS; DBP: 75.6 ± 7.7 vs 72.5 ± 9.4 mmHg - NS; MAP: 93.9 ± 9.2 vs 89.8 ± 9.4 mmHg - NS; PP: 50.9 ± 17.2 vs 52.2 ± 7.7mmHg - NS). The median QTC interval stands respectively (MA+ vs C) at 409 vs 396 mm/s; the proportion of patients with QTC interval longer than 430 mm/s is 14.3 vs 0%.

Conclusion: The frequency of SAS in T1D patients is high and this population often suffers from macroangiopathies. The study of T1D with SAS and macroangiopathy underlines a high frequency of associated neuropathy. The comparison between this group and a control group with no SAS and no macroangiopathy shows no difference in usual blood pressure characteristics. On the contrary, the study of QT (and particularly QTC) interval shows a significant lengthening. Referring to its potential implication in rhythm damage, it has to be systematically assessed in this context.

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Presence of type 2 diabetes mellitus significantly modulates the power of thyroid stimulating hormone to predict cardiovascular mortality

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Background and aims: Elevated thyroid stimulating hormone (TSH) is associated with an adverse cardiovascular risk profile, especially in patients with type 2 diabetes (T2DM). We investigated the association between TSH and cardiovascular mortality in patients with T2DM as well as in non-diabetic subjects.

Materials and methods: We measured TSH in a high-risk cohort of 1741 consecutive patients undergoing coronary angiography for the evaluation of established or suspected coronary artery disease (CAD). The incidence of vascular events was recorded over 10 years; T2DM was defined according to current ADA criteria.

Results: From our patients, 34% suffered vascular events. TSH proved to be a strong and independent predictor of cardiovascular mortality in subjects without T2DM (n=1220; standardized adjusted hazard ratio (HR) 1.11 [1.00-1.24]; p=0.036), but not in patients with T2DM (n=521; HR 0.99 [0.87-1.14]; p=0.934). An interaction term TSH x T2DM was significant (p=0.039), indicating that TSH was a significantly stronger predictor of vascular events in subjects without T2DM than in patients without T2DM.

Conclusion: From the data of this prospective cohort study we conclude that presence of T2DM significantly modulates the power of TSH to predict cardiovascular mortality.

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Is it possible to improve the prediction of silent myocardial ischaemia in type 1 diabetes?

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Background and aims: Cardiovascular disease (CVD) is the major cause of mortality in type 1 diabetes (T1DM). Given the higher prevalence of silent

myocardial ischemia (SMI) in this population, the need to screen asymptomatic T1DM patients assumes increasing importance. The aims of this study were: 1) to assess the prevalence of SMI in asymptomatic patients with T1DM, 2) to evaluate the clinical predictors associated with its presence, including the evaluation of microvascular complications and the assessment of arterial stiffness (AS) (as a measurement of pre-clinic arteriosclerosis) and 3) to evaluate the diagnostic value of the previous established criteria for its diagnosis (ADA 1998) and how to improve them.

Materials and methods: 80 patients with T1DM (without previous history of CVD) were screened for SMI by stress myocardial perfusion gated-SPECT (Single Photon Emission Computed Tomography). Data on traditional cardiovascular risk factors and microvascular complications were also recorded. AS was assessed by aortic pulse wave velocity (aPWV - Sphygmocor®).

Results: The baseline characteristics of the patients included in the study are shown in Table 1. The prevalence of SMI was 12.5%. SMI was associated with the presence of hypertension (OR 4.5 (95%CI 1.1–18.9); $p=0.041$) and HbA1c value (OR 2.4 (95%CI 1.1–5.0); $p=0.023$). The ADA criteria for the screening of SMI showed a good predictive value (OR 8.3 (95%CI 1.7–40.6); $p=0.009$, AUC 0.73 (0.54–0.91)). However, the addition of HbA1c value (AUC 0.87 (0.75–0.99) vs. 0.73 (0.54–0.93); $p=0.034$) and the presence of diabetic retinopathy (AUC 0.86 (0.74–0.98) vs. 0.73 (0.54–0.92); $p=0.057$) improved the predictive value, thus resulting in the best model of SMI prediction (AUC 0.91 (0.83–0.99) vs. 0.73 (0.54–0.92); $p=0.026$). aPWV did not add any significant improvement for SMI identification ($p=0.583$).

Conclusion: The prevalence of SMI in patients with T1DM without previous CVD is 12.5%. Although The ADA criteria for the screening of SMI predicted its presence adequately, adding HbA1c and taking into account the presence of diabetic retinopathy significantly improve the prediction rate.

Table 1. Clinical characteristics of patients with type 1 diabetes.

Clinical characteristics	Total (n=80)	No SMI (n=70)	SMI (n=10)	p
Age (yrs.)	49.7 (42.5–56.3)	49.7 (42.5–56.3)	50.7 (44.4–55.2)	0.860
Gender (male/female) n (%)	40/40 (50/50)	33/37 (47.1/52.9)	7/3 (70/30)	0.171
Smoking, n (%)	26 (32.91)	20 (28.6)	6 (60)	0.155
Family history of premature cardiovascular disease, n (%)	13 (16.25)	11 (15.7)	2 (20)	0.737
Hypertension, n (%)	31 (38.75)	24 (34.3)	7 (70)	0.032
Dyslipidaemia, n (%)	54 (67.5)	46 (65.7)	8 (80)	0.349
Diabetes				
Diabetes duration (yrs.)	19 (15–28)	20 (15–27)	18.5 (13–33)	0.976
Microvascular complications, n (%)	40 (57.1)	34 (48.6)	6 (60)	0.265
Retinopathy, n (%)				0.237
None, n (%)	53 (66.25)	48 (68.6)	5 (50)	
Non-proliferative, n (%)	12 (15)	11 (15.7)	1 (10)	
Proliferative, n (%)	15 (18.75)	11 (15.7)	4 (40)	
Nephropathy, n (%)	26 (38.24)	21 (30)	5 (50)	0.139
Peripheral neuropathy, n (%)	4 (5.06)	3 (4.3)	1 (10)	0.490
Anthropometric measurements				
BMI (kg/m ²)	26.4 (24.0–28.2)	26.4 (24.1–29.2)	25.3 (21.7–27.2)	0.512
Waist-to-hip ratio	0.91 (0.86–0.96)	0.91 (0.85–0.96)	0.91 (0.90–1.02)	0.235
Systolic blood pressure (mmHg)	126.3 (12.5)	125.2 (11.9)	133.8 (14.7)	0.041
Diastolic blood pressure (mmHg)	71.8 (9.3)	71.6 (8.8)	73.6 (12.8)	0.524
Mean arterial pressure (mmHg)	90.4 (9.4)	89.4 (8.8)	93.7 (12.9)	0.189
Laboratory parameters				
Fasting plasma glucose (mg/dl)	134 (90–196)	126 (90–195)	150 (139–213)	0.288
HbA _{1c} (%)	7.8 (7.1–9.4)	7.7 (7.1–8.6)	8.3 (7.9–9.4)	0.017
Total cholesterol (mg/dl)	184 (163–202)	184 (163–200)	184 (176–212)	0.325
HDL-cholesterol (mg/dl)	69.2 (55.8–88)	70.75 (55.8–88.1)	66.7 (56.9–74.2)	0.511
LDL-cholesterol (mg/dl)	97 (84–111)	93.5 (81–110)	102 (97–121)	0.126
Triglycerides (mg/dl)	64 (52–74)	63.5 (51–74)	68 (58–73)	0.468
Arterial stiffness				
aPWV (m/s)	7.75 (6.98–8.78)	7.85 (7.10–8.75)	8.05 (6.80–9.40)	0.971

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Integrative analysis of biomarkers for cardiovascular complications through networks of metabolites and proteins

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Background and aims: The SUMMIT project (SURrogate markers for Micro- and Macro-vascular hard endpoints for Innovative diabetes Tools), seeks to identify genetic and non-genetic biomarkers for prediction of disease progression and risk of vascular complications. A collection of samples from different cohorts have been provided by combining a variety of high-throughput techniques encompassing proteomics and metabolomics. Integrative bioinformatics methods that combine different data sources may provide deeper

insights into the molecular interactions underlying complex diseases. Within this context, we constructed a network with proteins and metabolites as nodes whose analysis suggests the most relevant entities involved in the disease pathways.

Materials and methods: Starting from a list of SUMMIT-discovered biomarkers, i.e., proteins and metabolites that have been shown to be associated with cardiovascular complications we obtained a disease-related network by combining evidences from different repositories. In detail, STRING, STITCH and MetaCore were used to initially build individual networks that were subsequently merged into a combined network associating the discovered markers to other molecules known to interact with them. Topological analysis of this network has been used to identify enriched biological pathways and mechanisms, and to suggest a set of relevant nodes as candidates for pharmacological therapies.

Results: The network representation could elucidate molecules and interactions involved in the disease and better define its global picture. While the different repositories can be used separately to build disease networks, merging these networks provided a better model of the whole disease context. The list of pathways identified as significantly enriched (p -value < 0.001) contained those related to insulin resistance as well as others that have not previously been identified as potential targets for standard diabetes therapies. For example, the signaling by Insulin-like Growth Factor 1 Receptor (IGF1R) was amongst those identified. This receptor is a negative regulator of insulin signaling and currently has not been taken into account for diabetes therapies. However, it has been shown that cardiac overexpression of IGF-1R in mice prevents diabetes-induced cardiac fibrosis and diastolic dysfunction.

Conclusion: We believe that higher integration across different systems biology platforms eases interpretation of discoveries, supports validation of hypothesis and ultimately speeds up the drug discovery process.

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Correlation of inflammatory markers and insulin resistance indexes with the short- and long-term outcome after an acute coronary syndrome in different glycaemic categories

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Background and aims: The glucose metabolism disorder is frequent in patients who are admitted after an Acute Coronary Syndrome (ACS). The aim of this study is to examine the correlation of ACS outcome with insulin resistance indexes and inflammatory markers in known diabetes patients, newly diagnosed diabetes patients, patients with impaired glucose tolerance (IGT) and normoglycaemic patients.

Materials and methods: 536 patients who were admitted at the Cardiology Department within a two-year period were included in the study. All patients underwent a clinical laboratory testing including insulin level in order to measure insulin-resistance indexes (HOMA- Homeostasis Model Assessment and QUICKI- Quantative Insulin Sensitivity Check Index) and inflammatory markers (high-sensitivity C-reactive protein (hs-CRP), white blood cells count (WBC), fibrinogen and erythrocyte sedimentation rate (ESR)). All non diabetic patients went under an oral glucose tolerance test (OGTT) one month after discharge. Study's end-points were death, a new ACS, arrhythmias and acute pulmonary oedema of cardiological origin during hospitalization and 12 months after the ACS.

Results: 199 (37,12%) patients were normoglycemic while 168 (31,34%) were known diabetics, 59 (11%) newly diagnosed diabetic patients and 110 (20,52%) were IGT patients. There was a statistically significant correlation of the hs-CRP with the outcome during hospitalization (HR=1.712, 95%CI:1.193–2.458, $p=0.004$) for all patients. hs-CRP (HR=2.612, 95%CI:1.474–4.628, $p=0.001$) and HOMA index (HR=1.967, 95%CI:1.184–3.267, $p=0.009$) were significantly correlated with the appearance of a major cardiovascular event (MACE) during hospitalization for all patients but only hs-CRP was correlated with MACE for each group separately. Complications during the first 12 months after an ACS were significantly correlated only with the hs-CRP for all patient categories. hs-CRP, HOMA and QUICKI indexes were correlated with MACE in a multivariate analysis for known diabetes patients (HR=1.542, $p=0.023$, HR=1.482, $p=0.039$ and HR=0.810,

$p=0.031$ respectively), newly diagnosed diabetes patients (HR=2.401, $p=0.035$, HR=1.364, $p=0.046$, HR=0.832, $p=0.041$) and IGTs (HR=1.354, $p=0.023$, HR=1.269, $p=0.047$, HR=0.782, $p=0.036$) 12 months after the ACS. Association of hs-CRP (HR=2.012, $p=0.008$), HOMA (HR=2.438, $p=0.002$) and QUICKI (HR=0.503, $p=0.010$) with MACE was significant in an univariate analysis while only hs-CRP (HR=1.477, $p=0.032$) remained significant in a multivariate analysis for normoglycaemic patients

Conclusion: hs-CRP is the inflammation marker which is correlated with the appearance of complications during hospitalization and 12 months after an ACS, while HOMA and QUICKI indexes are correlated with the appearance of major cardiovascular events during hospitalization as well as the 12-month follow-up period regardless of patient glycaemic profile. The combination of these indexes with hs-CR significantly improves the latter's prognostic model in particular regarding major cardiovascular events.

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Both DHA/AA ratio and absolute DHA levels, but not EPA levels, constitute an independent risk factor for the severity of coronary atherosclerosis in type 2 diabetic patients

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Background and aims: The concentrations of dietary n-3 polyunsaturated fatty acids (PFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) modify the incidence of coronary heart disease (CHD). Especially, the effects of EPA on cardiovascular events have been clarified through recent large-scale intervention clinical studies. However, it is still unknown whether DHA may make a contribution to clinical efficacy associated with the risk of CHD. Thus, we investigate which n-3 PFA is more effective on the association of the severity of CHD in type 2 diabetic patients.

Materials and methods: 184 type 2 diabetic subjects were nominated as poor blood glucose control from April 2008 to September 2010 and aged 40 to 72 years on this trial. We firstly calculated fasting plasma PFAs, LDL-cholesterol, HDL cholesterol and triglyceride (TG) levels. The subjects were asked about daily intake of different type of fish and n-3 PUFAs intake was calculated using computer food composition software. We excluded the subjects who reported the history of CHD or ECG abnormality consistent with ischemia at baseline survey. Then they had been followed for five years, focusing on the occurrence of major cardiovascular events or ECG abnormality for an observation period, which compromised nonfatal and fatal CHD diagnosed using percutaneous coronary intervention. CHD severity was assessed using the Gensini scoring system. Next, we divided them into 2 groups, who were in the presence of CHD (DM-PCI, N=56) and no episodes of CHD (DM, N=70) and 71 healthy volunteers were nominated (NGT). These values were analyzed using an ANOVA model, Pearson's test and multivariate logistic regression models analyses.

Results: The ratios of smokers and subjects with antihypertensive and/or lipid modifying treatment were not significant between DM-PCI and DM. The average of systolic blood pressure, age, BMI, the concentrations of HbA1c, LDL, HDL and TG were no different between two groups. On the contrary, the concentrations of FPG, HbA1c, LDL, TG and remnant like particle (RLP) were significantly lower in NGT than in DM-PCI and DM. Firstly, we investigated the relation between the basal plasma concentrations of both EPA and DHA and daily fish intake was the positive correlation in the diabetic subjects ($r=0.77$ $p<0.001$, $r=0.61$ $p<0.001$, respectively). These data indicated the plasma concentrations of PFAs were considered to originate from fish intake. Both EPA/AA and DHA/AA levels in DM-PCI were significantly lower than those in NGT (EPA/AA; 0.31, 0.47 $p<0.001$, DHA/AA; 0.69, 0.85 $p<0.001$, respectively) but not significant compared to DM. Although the concentrations of both EPA/AA and DHA/AA in DM-PCI were significantly negative correlation to Gensini score ($r=0.44$ $p<0.001$, $r=0.50$ $p<0.001$, respectively), there were no correlation between this score and non-HDL, LDL/HDL, RLP, HbA1c. In multivariate logistic regression model, only DHA/AA ratio increased up to 3.08 (95% CI 1.76–5.89) with statistical value of $p=0.01$.

Conclusion: A negative correlation was noted between the severity of CHD and DHA/AA ratio in DM-PCI, as same as the absolute values for DHA. In addition, the lower levels of DHA/AA in DM-PCI may show us the evidence that PFAs, especially DHA intake, was inversely and independently associated the severity of CHD.

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Association of NLRP3 inflammasome activation and cardiovascular disease risk using Framingham score in patients with newly diagnosed type 2 diabetes mellitus

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Background and aims: Chronic low-grade inflammation caused by activation of the innate immune system plays an essential role in the pathogenesis of type 2 diabetes and its major complications. Emerging evidence suggests that activation of the nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome leads to the maturation and secretion of interleukin (IL)-1 β and is involved in the pathogenic mechanisms of obesity-induced inflammation, insulin resistance, and type 2 diabetes development. In our previous study, NLRP3 inflammasome activation is elevated in myeloid cells from drug-naïve, newly diagnosed type 2 diabetic patients and antidiabetic treatment with metformin contributes to modulation of inflammasome activation. These previous reports prompted us to explore the association of cardiovascular risk and the inflammasome activation in type 2 Diabetes.

Materials and methods: We performed a sub-analysis to evaluate the association of cardiovascular risk using the Framingham cardiovascular risk score with the NLRP3 inflammasome activation in 47 diabetic patients.

Results: Patients had a male-to-female ratio of 31:16, mean age of 51.9 ± 11.8 years, a mean body mass index of 25.4 ± 3.0 kg/m², a mean HbA1c of 8.5 ± 2.3 %, a mean low density lipoprotein-cholesterol of 140 ± 37 mg/dl, and a mean baseline Framingham risk score of 7.3 ± 6.7 %. Framingham risk score was associated neither relative mRNA expression of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC) nor production of proinflammatory cytokines regardless of stimulation with lipopolysaccharide (LPS) in monocyte-derived macrophages (MDMs) from the type 2 diabetic patients (Table 1). Also, Framingham score was not correlated with IL-1 β secretion in MDMs after stimulation with various danger molecules such as adenosine triphosphate, monosodium uric acid crystals, islet amyloid polypeptides, and free fatty acids. The type 2 diabetic patients with intermediate to high cardiovascular risk (Framingham risk score $>10\%$) did not show the increase of either IL-1 β or IL-18 in serum compared to the patients with low risk (Framingham risk score $<5\%$, 23.3 ± 11.5 vs 21.3 ± 16.7 pg/ml; $P=0.651$, 34.6 ± 14.3 vs 31.2 ± 16.5 pg/ml; $P=0.530$).

Conclusion: In conclusion, the NLRP3 inflammasome activation was not associated the cardiovascular risk in the newly diagnosed type 2 diabetes.

Table 1. Association inflammasome activity with Framingham cardiovascular risk score.

	<i>r</i>	<i>P</i>
IL-1 β mRNA_untreated	-0.060	0.073
IL-1 β mRNA_LPS	0.007	0.965
NLRP3 mRNA_untreated	0.026	0.870
NLRP3 mRNA_LPS	0.018	0.911
ASC mRNA_untreated	0.122	0.435
ASC mRNA_LPS	-0.092	0.557
IL-1 β secretion_untreated	-0.021	0.894
IL-1 β secretion_LPS	0.082	0.599
IL-18 secretion_untreated	0.048	0.759
IL-18 secretion_LPS	0.078	0.619
IL-1 β _serum	0.046	0.768
IL-18_serum	-0.059	0.707

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Soluble urokinase plasminogen activator receptor is elevated in diabetes and associated with diabetic complications in patients with type 1 diabetesS. Theilade¹, S. Lyngbaek², T.W. Hansen¹, J. Eugen-Olsen³, M. Fenger⁴, P. Rossing^{1,5}, J. Jeppesen^{2,5};¹520, Steno Diabetes Center, Gentofte, ²Department of Medicine, Glostrup Hospital, Glostrup, ³Clinical Research Centre, Hvidovre Hospital,⁴Department of Biochemistry, Hvidovre Hospital,⁵Aarhus University, Denmark.

Background and aims: Soluble urokinase plasminogen activator receptor (suPAR) is a marker of inflammation and endothelial dysfunction. We investigate associations between suPAR and diabetic complications in type 1 diabetes.

Materials and methods: From 2009–2011, 667 type 1 diabetes patients and 51 non-diabetic control subjects were included in a cross-sectional study at our center. suPAR was measured with ELISA (ViroGates, Denmark). Diabetic complications were cardiovascular disease (CVD) (previous myocardial infarction, revascularisation, peripheral arterial disease and stroke), autonomic dysfunction (heart rate variability during deep breathing < 11 beats per minute), albuminuria (urinary albumin excretion rate (UAER) \geq 30 mg/24-hours) or high arterial stiffness (pulse wave velocity \geq 10 m/s). Adjusted analyses included gender, age, systolic blood pressure, estimated glomerular filtration rate, UAER, HbA_{1c}, total-cholesterol, body mass index, C-reactive protein, antihypertensive treatment and smoking.

Results: suPAR was lower in controls vs. patients, controls vs. normoalbuminuric patients (< 30 mg/24-hours), normoalbuminuric patients with short vs. long diabetes duration (> 10 years), and increased with degree of albuminuria (adjusted $p < 0.001$ for all). Furthermore, suPAR levels were higher in patients with vs. without CVD ($n = 144$ (21.3%)), autonomic dysfunction ($n = 349$ (59.2%)), albuminuria ($n = 357$ (53.1%)) and high arterial stiffness ($n = 297$ (47.2%)) (adjusted $p \leq 0.024$). Per 1 unit increase in ln-suPAR adjusted odds ratios were for CVD: 2.5 (1.1–5.7), autonomic dysfunction: 2.7 (1.2–6.2), albuminuria: 3.8 (1.3–10.9) and high arterial stiffness: (2.5 (1.1–6.1) ($p \leq 0.039$)).

Conclusion: suPAR is associated with type 1 diabetes, diabetes duration and complications independently of other risk factors. suPAR may be a novel risk marker in the management of diabetes.

Clinical Trial Registration Number: 2009-056; NCT01171248

PS 112 Clinical observations in children

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The role of glucagon in post-prandial hyperglycaemia for children 5 years after onset of type 1 diabetesS. Fredheim^{1,2}, M.L.M. Andersen¹, S. Pørksen¹, L.B. Nielsen¹, C. Pipper³, L. Hansen¹, J. Johannesen^{1,2}, H.B. Mortensen^{1,2}, J. Svensson^{1,2};¹Pediatric Department, Herlev Hospital, ²Copenhagen University,³Department of Biostatistics, Copenhagen University, Denmark.

Background and aims: The role of glucagon on glycemic control in type 1 diabetes is debated, and high glucose concentrations have previously been shown to increase glucagon release. We investigated stimulated glucagon and glucagon-like peptide-1 (GLP-1) levels in children during 12 to 60 months after onset of type 1 diabetes.

Materials and methods: The study cohort comprised 129 children (66 boys) mean (SD) age at onset; 10.04 (3.9) range 0.6–16.6 years with newly diagnosed type 1 diabetes. Liquid mixed-meal (Boost) stimulated levels of C-peptide, glucagon, glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), blood glucose (measured 90 min post-ingestion of Boost drink), and HbA_{1c} (DCCT), were analysed centrally 1, 3, 6, 12 and ($N=40$) 60 months (m) after diagnosis. Robust multivariate regression methods were used for statistical analyses. Results are listed as RR [95% CI] corresponding to a doubling of the explanatory variable. Analyses were adjusted for age, sex, diabetes duration and stimulated C-peptide.

Results: During the 60 months, post-prandial glucagon levels were highly associated to the rise in stimulated GLP-1 (RR 1.33 [1.19; 1.48], $p < 0.0001$) and stimulated blood glucose levels (1.36 [1.19; 1.56], $p < 0.0001$). Post-prandial glucagon was negatively associated to stimulated C-peptide at 3 (RR 0.94, [0.89; 0.99], $p = 0.019$), 6 (RR 0.91 [0.87; 0.95], $p < 0.0001$) and 12 months ((RR 0.94, [0.90; 0.99], $p = 0.021$), when residual beta-cell function is present), but no association existed 60 months post-diagnosis. HbA_{1c} associated significantly to post-prandial glucagon levels (RR 1.03, [1.01; 1.05], $p = 0.003$). A doubling of post-prandial glucagon levels corresponded to a 3% relative increase in HbA_{1c} levels. Furthermore, HbA_{1c} levels were negatively associated to stimulated C-peptide at 3 ($p < 0.0001$), 6 ($p < 0.0001$) and 12 months ($p < 0.0001$), but not 60 months post-diagnosis.

Conclusion: Post-prandial glucagon levels were highly associated with worsening of glycaemic control and blood glucose levels during the first 60 months after diagnosis. This suggests that elevated glucagon augmented hyperglycaemia (HbA_{1c}) observed in the time period. The positive association with GLP-1 suggests that physiologic levels of GLP-1 levels are insufficient to constrain glucagon.

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Disease management and outcomes in European children, adolescents, and young adults with type 1 diabetes (T1D): the global TEENs studyM. Phillip¹, L. Laffel², C. Domenger³, V. Pilorget⁴, C. Candelas⁴, T. Danne⁵, C. Mazza⁶, B. Anderson⁷, R. Hanas⁸, S. Waldron⁹, R. Beck¹⁰, C. Mathieu¹¹;¹Schneider Children's Medical Center of Israel, Petah Tikva, Israel,²Joslin Diabetes Center, Boston, USA, ³Sanofi, Paris, ⁴Sanofi, ChillyMazarin, France, ⁵Kinder und Jugendkrankenhaus "Auf der Bult",Hannover, Germany, ⁶Hospital de Pediatría J P Garrahan, Buenos Aires,Argentina, ⁷Baylor College of Medicine, Houston, USA, ⁸GothenburgUniversity, Sweden, ⁹Hosted by West Midlands Strategic Clinical Network,Birmingham, UK, ¹⁰Jaeb Center for Health Research, Tampa, USA,¹¹University Hospitals (UZ), Leuven, Belgium.

Background and aims: TEENs is the largest contemporary, international, cross-sectional study of young T1D patients ($N=5,960$), aiming to identify approaches to optimise glycaemic control and outcomes. Data from the European cohort ($n=2,943$; 49.4% of the global population) are reported.

Materials and methods: Of the 219 TEENs centres from 20 countries, 111 centres from 11 European countries (Denmark, France, Germany, Hungary, Italy, Portugal, Romania, Russia, Slovenia, Spain and Sweden) participated. Data were collected by patient interview, medical record review, and patient/parent survey from three age groups: children (8–12 years old [y/o]), adolescents (13–18 y/o), and young adults (19–25 y/o). A1c was measured uniformly (reference range 4–6%) using A1cNow™ (Bayer). Target A1c levels were cat-

egorised as <7.5% (58 mmol/mol) for patients ≤18 years of age (ISPAD) and <7% (53 mmol/mol) for patients 19–25 years of age (ADA). Patients aged 13–18 and 19–25 y/o completed the Problem Areas In Diabetes (PAID) questionnaire; parents of youth aged 8–12 and 13–18 y/o completed the PAID parent-revised (PAID-PR) version. Scores on each survey range from 0 to 100; higher scores indicate greater disease burden.

Results: Median (interquartile range) time since T1D diagnosis was 6.5 years (3.7–9.9). Overall mean (±SD) A1c was 8.1±1.6% (65±18 mmol/mol), with 35% of patients attaining A1c targets. Treatment characteristics and diabetes management methods by age group and A1c target attainment as well as treatment burden by age group are shown (Table).

Conclusion: In a sample of 8–25-year-olds with T1D from 11 European countries, A1c was suboptimal and approximately one third of patients achieved targets. In the TEENs study, European patients at target tended to receive more intensive management than those not at target, especially patients aged 8–18. Parents/guardians reported a significantly higher perceived burden of care compared with adolescents. Thus, opportunities exist to improve A1c outcomes and reduce burden of disease for youth with T1D.

	European subgroup		
	8–12 years n=887	13–18 years n=1,451	19–25 years n=605
At target			
N (%)	349 (39)	529 (36)	137 (23)
BG monitoring times/day, mean (SD)	6.2 (2.3)	5.1 (1.9)	3.9 (2.0)
CGM use in last month, n (%)	19 (5)	24 (5)	6 (4)
Glucose available at home, n (%)	299 (86)	444 (84)	101 (74)
Use of carbohydrate counting for diabetes management, n (%)	188 (54)	261 (49)	66 (48)
Insulin device, n (%)			
Pump	117 (34)	183 (35)	33 (24)
Injectors/pens*	231 (66)	343 (65)	103 (75)
Not at target			
N (%)	537 (61)	922 (64)	468 (77)
BG monitoring times/day, mean (SD)	5.5 (1.8)	4.4 (1.7)	3.7 (1.9)
CGM use in last month, n (%)	26 (5)	31 (3)	8 (2)
Glucose available at home, n (%)	400 (74)	655 (71)	283 (62)
Use of carbohydrate counting for diabetes management, n (%)	270 (50)	406 (44)	230 (49)
Insulin device, n (%)			
Pump	155 (29)	253 (27)	114 (24)
Injectors/pens*	380 (71)	666 (72)	354 (76)
PAID scores			
PAID, mean (SD)		23.6 (18.0)	26.1 (18.3)
PAID-PR, mean (SD)	47.6 (20.2)	47.1 (20.5)	

*Any treatment type; BG, blood glucose; CGM, continuous glucose monitor

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High and increasing prevalence of diabetic ketoacidosis at diagnosis of type 1 diabetes in children, Colorado, 1998–2012

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Background and aims: Diabetic ketoacidosis (DKA) at the time of diagnosis of childhood diabetes is life-threatening and has a detrimental effect on the future course of the disease. Frequency of DKA reflects access to care and indicates the level of children's health in a country. The aim of this study was to analyze time trends in DKA at diagnosis of childhood diabetes in Colorado during 1998–2012 and to explore modifiable risk factors.

Materials and methods: A prospective registry of children diagnosed with diabetes before 18 y of age during 1998–2012 and treated at the Barbara Davis Center for Childhood Diabetes in Denver, CO. A total of 4989 children were registered, including 3544 diagnosed with type 1 diabetes and residing in Colorado at the time of diagnosis. The main outcome measure was prevalence of DKA at the time of diagnosis of diabetes.

Results: The prevalence of DKA increased significantly from 29.9% in 1998 to 46.2% in 2012 ($p<.001$ for trend); the most rapid increase occurred between 2007 and 2012. DKA was most likely to occur at diagnosis among the youngest children and those of African-American ancestry. Family history of diabetes, especially type 1 diabetes among 1st degree relatives, was protective as was participation in research studies following children at high genetic risk. Coverage by a private health insurance (OR=0.37; 0.29–0.49) or a government-provided one (OR=0.63; 0.51–0.81), was associated with a lower likelihood of DKA, compared to no insurance. The number of children covered by government-provided health insurance has doubled and their DKA rates continue to be high. In addition, DKA is rapidly increasing among children with private health insurance.

Conclusion: The rapid and unabated increase in the prevalence of DKA at diagnosis of childhood type 1 diabetes suggests a major worsening in early recognition of diabetic symptoms and also in access to appropriate health care among Colorado children over the past 15 years. The frequency of DKA has increased to the levels rarely seen in developed countries.

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Diabetic ketoacidosis (DKA) in children with type 1 diabetes mellitus (T1DM): an Italian multicentre survey

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Background and aims: Italian children with T1DM are usually followed by pediatric diabetologists. Since recent data on epidemiology and management of primary diabetic ketoacidosis in children with T1DM are lacking a questionnaire was sent to the 77 centres belonging to the study group of diabetology of the Italian Society for Pediatric Diabetology and Endocrinology (ISPED) enquiring on epidemiology (2012–2013) and present management of DKA.

Materials and methods: From 1/1/2012 to 31/12/2013 a survey on DKA was conducted in all pediatric Centres belonging to the ISPED. Presence DKA was defined according to the IDF/ISPAD criteria. The following data were collected: treatment according IDF/ISPAD protocol yes or not, type of rehydrating solution used, bicarbonates use yes or not and amount of insulin infused.

Results: Data were returned from 68/77 Centres (87%) for a total of 14,493 patients with T1D. We recorded 2453 children with T1DM onset, with DKA in 945 (38.5%) (severe in 10.3%). Considering only preschool children DKA was observed in 72% (severe in 16.6%). Cerebral oedema following DKA treatment was observed in 5 cases (0.5%). DKA treatment according ISPAD guidelines was adopted in 67% of the Centres, while 11% did not follow any specific guidelines. In the first 1–2 hours, rehydration was started with normal saline, at different rates: 5–10 ml/kg/h in 71%, 10–20 ml/kg/h in 16%, <5 ml/kg/h in 4%. After the first hours, differences among Centres were observed regarding the type of solutions used: saline 0.9–0.45% in 75%, 5–10% glucose solution in 19%, irrespective of glycemic values. Potassium supplementation was performed at the rate of 20–40 mEq/l in 63% of Centres. Bicarbonates were never used in 17% of Centres, while in 68% were exceptionally used according to pH and clinical conditions. Insulin was infused starting from 2nd–3rd hour at the rate of 0.05–0.1 U/kg/h in 63% of Centres, while others used infusion rate lowest as 0.025 U/kg/h.

Conclusion: Notwithstanding prevention campaign, DKA is still observed at clinical diabetes onset in Italian children. Despite international guidelines significant variability in DKA treatment still exists, underlying the need to share them among Centres. In Italy cerebral edema is a rare complication of DKA.

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Investigation of cerebrovascular changes in type 1 diabetic children and adolescents with transcranial Doppler ultrasound

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Background and aims: It is a well-known fact that diabetes (both type 1 and type 2) play a significant role in developing ischemic stroke in adults. Despite the fact that the prevalence of type 1 diabetes is rising among children and adolescents very few attention is paid to the cerebrovascular complication caused by this disease. Our aim was to measure the possible effect of dia-

betes on the cerebrovascular system with a simple, non-invasive method the transcranial Doppler sonography.

Materials and methods: We performed transcranial Doppler sonography in 62 type 1 diabetic patients (older than 10 years of age, mean 14.59 ± 2.46 years) treated in our hospital and 46 (mean age 13.66 ± 2.06) healthy children served as controls. We examined the middle cerebral arteries in both sides in lying position during rest by registering the velocities (peak systolic and end diastolic). Then we asked the children to stop their breathing as long as they can (but at least for 30 seconds) to increase the PCO₂ which led to vasodilatation. After a short period of resting period came the decreasing of PCO₂ (causing vasoconstriction) by asking the children to take deep breaths for 30 seconds. After collecting these data we calculated the pulsatility index ((systolic velocity-diastolic velocity)/mean velocity) and resistive index ((systolic velocity-diastolic velocity)/systolic velocity) in all the 3 cases.

Results: The Kolmogorov-Smirnov test showed that all of the investigated variables showed normal distribution thus two-tailed t tests could be performed to find statistical differences between diabetic patients and control group. After processing the data we can conclude that the diabetic group showed lower values in all investigated variables. The strongest difference ($p=0.0214$) could be found in the peak systolic velocities after hyperventilation (82.8 cm/s vs. 91.8 cm/s). An other significant ($p=0.0401$) difference was detected in the peak systolic velocities after hypoventilation (136.6 cm/s vs. 146.5 cm/s), while all other findings proved to be non-significant.

Conclusion: The fact that all measured velocities were lower among diabetic children and adolescents suggests that some kind of pathophysiological changes must have been started among these patients and this harmful influence would proceed and would be responsible for the further well-known effects among adults. We assume that the first phase of this process affects the response for the „CO₂ challenge“, that's why the peak systolic velocities are influenced after both hypo- and hyperventilation. Besides raising the number of performing transcranial Doppler sonography we are working on identification of factors which can play a role forming the changes among our patients.

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40 year follow up of childhood onset diabetes reveals little long-term decline in the cumulative incidence of complications by diagnosis cohort except for renal failure

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Background and aims: Despite dramatic improvements in life expectancy, morbidity rates in type 1 diabetes (T1D) still greatly exceed those of the general population. To evaluate trends, by year of diagnosis, in the cumulative incidence of micro- and macro-vascular complications over 22 years of follow-up, the prospective, observational, Pittsburgh Epidemiology of Diabetes Complications (EDC) study of childhood-onset T1D study was examined.

Materials and methods: Participants were categorized into five calendar year onset cohorts: 1950-59, 1960-64, 1965-69, 1970-74, and 1975-80. Renal failure (RF, self-report), hard coronary artery disease (CAD; MI, fatal CAD and revascularization), blindness (self-report) and amputations (self-report) were determined at 20, 25, 30, 35 and 40 years duration on the complete cohort ($n=912$). Proliferative retinopathy (PR; fundus photography), confirmed distal symmetric polyneuropathy (CDSP; clinical exam plus vibratron threshold), symptomatic autonomic neuropathy (SAN; 2+ symptoms and expiration/inspiration ratio <0.1), Lower Extremity Arterial Disease (LEAD; Ankle Brachial Index <0.9 /amputation/intermittent claudication) and overt nephropathy (ON; albumin excretion rate >200 μ g/min), were assessed at 20, 25, 30, and 35 years' duration on the subset of participants with a clinical examination ($n=658$).

Results: There was a marked decline in the cumulative incidence of RF across diagnosis cohorts at all durations ($p<0.01$ - <0.001). An early decline in blindness in those more recently diagnosed at 20 years duration ($p<0.001$) was no longer apparent at 30 or 40 years duration ($p>0.6$). CAD showed a small decline at 30 years across cohorts ($p<0.03$) which was less marked at 40 years ($p=0.07$). Amputations, PR, LEAD, ON, CDSP and SAN showed no diagnosis cohort declines except for CDSP at 20 years alone ($p=0.04$).

Conclusion: These data suggest that the cumulative incidences of T1D complications are not declining universally although a strong decline in RF is apparent which may represent better management of renal disease rather than prevention.

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Type 2 diabetes and associated complications in Western Australian children: a population based study (1990 - 2012)

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Background and aims: Data on childhood diabetes are available from the Western Australian Children's Diabetes Database (WACDD), maintained by the only tertiary paediatric hospital in Western Australia servicing all children diagnosed with diabetes throughout the State. This study aimed to describe the incidence of type 2 diabetes in Western Australian children and examine the prevalence of complications of diabetes.

Materials and methods: The study included children aged 0-16 years diagnosed with type 2 diabetes at our hospital, between 1990 to 2012, in a population-based sample. Incidence rates per 100,000 person-years at risk were calculated by Indigenous status and year of diagnosis. Mean (\pm SD) were estimated for age, BMI Z-score and HbA1c at diagnosis. Prevalence of microalbuminuria and hypertension were evaluated for patients at different time points for all available complications screening data.

Results: From 1990 to 2012, there were 135 (82 F:53 M) incident cases of type 2 diabetes in children aged <17 years. Although Indigenous children make up $\sim 6\%$ of the general population, they were grossly over-represented, accounting for 56% of cases. The mean incidence of type 2 diabetes was 0.6 per 100,000 (95%CI: 0.5-0.8) in non-Indigenous children compared to 12.6 per 100,000 (95%CI: 10.0-15.8) in Indigenous children. The mean age at diagnosis was $13.3(\pm 2.0)$ years. At diagnosis, the mean BMI Z-score was $2.0(\pm 0.6)$, with 12% of cases classified as being overweight and 61% obese. Mean HbA1c at diagnosis was $9.0(\pm 2.8\%)$ compared to $7.7(\pm 2.5\%)$ 3-12 months post-diagnosis. Hypertension was observed in 15/75 (20%) of cases at the time of diagnosis and in 19% of cases 2 years post-diagnosis. Similarly, microalbuminuria was detected in 11/61 (18%) of cases at the time of diagnosis and 23% of cases 2 years post-diagnosis.

Conclusion: In keeping with other populations, the incidence of childhood type 2 diabetes is increasing in Western Australia, the highest incidence being observed in Indigenous children. Despite their youth, complications of diabetes were already present in this cohort at diagnosis and their prevalence increased markedly even with short duration of disease. There is presently no screening program for children at increased risk of developing type 2 diabetes in Western Australia, and older adolescents with type 2 diabetes may not be ascertained by the paediatric service. Therefore, undiagnosed cases are likely to exist in the study population, and these data represent a likely underestimation.

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Glycaemic control and acute complications in children, adolescents, and young adults with type 1 diabetes (T1D): the global TEENs study

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Background and aims: Optimal glycaemic control remains elusive for many young patients with T1D. The TEENs study is the largest contemporary, international, observational study of young T1D patients ($N=5,960$), aiming to identify approaches to optimise glycaemic control and outcomes.

Materials and methods: 219 centres from 20 countries collected data by patient interview, medical record review, and patient/parent survey from three age groups (8-12-year-olds [y/o], $n=1,724$), adolescents (13-18 y/o, $n=2,854$), and young adults (19-25 y/o, $n=1,382$). A1c was measured uniformly (reference range 4-6%) using A1cNow™ (Bayer). Target A1c levels were categorised as $<7.5\%$ (58 mmol/mol) for patients ≤ 18 years of age (ISPAD) and $<7\%$ (53 mmol/mol) for patients 19-25 years of age (ADA). Factors associated with attaining target A1c were identified by multivariate analysis.

Results: Median (interquartile range) duration of T1D was 6.1 (3.3–9.6) years; mean (\pm SD) A1c was $8.5 \pm 1.8\%$ (69 ± 20 mmol/mol). A1c targets were achieved by 28% of patients (32% of 8–12 y/o, 29% of 13–18 y/o, and 19% of 19–25 y/o). Overall, 351 (5.9%) patients reported diabetic ketoacidosis (DKA) in the past 3 months, with the highest occurrence in 19–25 y/o (6.6% vs 5.6% in 8–12 y/o and 5.7% in 13–18 y/o). Severe hypoglycaemia leading to seizure or loss of consciousness was reported by 162 (2.7%) patients with the highest occurrence in 19–25 y/o (4.1% versus 2.5% in 8–12 y/o and 2.2% in 13–18 y/o). Incidence of acute T1D complications by A1c goal attainment and age are shown (Table). In all age groups, occurrence of DKA rates was higher in those not at A1c target. Rates of microalbuminuria, retinopathy treatment, and neuropathy were highest in 19–25 y/o and in patients not at target. In multivariate analyses, presence of DKA (OR 0.53 [95% CI 0.39, 0.73]) and diabetic neuropathy symptoms (OR 0.47 [95% CI 0.34, 0.66]) were significantly associated with not attaining A1c target ($p < 0.001$).

Conclusion: In the TEENS study, occurrence of acute complications tended to be higher in patients not at target. Diabetes outcomes in these young T1D patients was suboptimal, with mean A1c above target for approximately two thirds of participants and many youth experienced acute complications, supporting the need for further improvements.

	8–12 years		13–18 years		19–25 years	
	A1c at target n=553* (32%)	A1c> target n=1,170* (68%)	A1c at target n=833 (29%)	A1c> target n=2,021 (71%)	A1c at target n=260 (19%)	A1c> target n=1,122 (81%)
Complications, n (%)						
Diabetic ketoacidosis	16 (2.9)	80 (6.8)	27 (3.2)	137 (6.8)	7 (2.7)	84 (7.5)
Severe hypoglycaemia	12 (2.2)	31 (2.7)	16 (1.9)	47 (2.3)	16 (6.2)	40 (3.6)
Microalbuminuria	6 (1.2)	14 (1.4)	18 (2.4)	90 (5.0)	14 (5.8)	88 (8.8)
Retinopathy treatment	0	1 (<0.1)	0	7 (0.3)	4 (1.5)	46 (4.2)
Neuropathy	8 (1.5)	32 (2.7)	17 (2.1)	116 (5.8)	21 (8.2)	111 (10.0)

*Data not available for one patient

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PS 113 Clinical observations in children and women

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Recent trends in type 1 diabetes incidence in children and adolescents in Germany

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Background and aims: Recent data from Nordic European countries suggest that the increase in the incidence of childhood type 1 diabetes (T1D) may have levelled off or the trend may have even reversed in recent years. Aim of the present study was to estimate the recent time trend in T1D among children and adolescents 0–19 years of age in the German federal state North Rhine-Westphalia (NRW) between 2002 and 2012.

Materials and methods: The average risk population consisted of 3.08 million children and adolescents. The NRW diabetes incidence register ascertains newly diagnosed cases of T1D by means of three data sources: the prospective hospital-based active surveillance system ESPED, annual inquiries among medical practices, and the DPV database, a computer-based documentation system for quality control and scientific research in diabetes care. Completeness of ascertainment was estimated by the capture-recapture-method. Point and interval estimates (95%CI) of incidence rates (per 100,000 person-years) were based on Poisson distribution. Age- and/or sex-standardized rates were estimated by the direct method using equal weights. Poisson regression analysis was applied to assess time trends adjusted for annual completeness of ascertainment.

Results: Between 2002 and 2012, a total of 8,274 children and adolescents (4,465 boys) aged 0–19 years with new-onset T1D were registered. Ascertainment was estimated to be 98.7% (95%CI: 98.4%; 99.0%) complete. The overall incidence rate was 20.8 (20.3; 21.2). The incidence among boys (21.8 (21.2; 22.5)) was higher than among girls (19.8 (19.1; 20.4), rel. risk 1.11 (1.07; 1.16), $p < 0.001$), mainly owing to the male preponderance among the age groups 10–14 (30.8 (29.4; 32.3) vs. 24.6 (23.3; 26.9)) and 15–19 years (11.4 (10.6; 12.3) vs. 8.3 (7.6; 9.1)). Age-specific estimates for age groups 0–4, 5–9, 10–14, and 15–19 years were 17.7 (16.8; 18.5), 28.0 (26.9; 29.0), 27.7 (26.7; 28.7), and 9.9 (9.3; 10.5), respectively ($p < 0.001$). The annual incidence rates ranged between 17.6 in 2002 and 23.3 in 2012. The average relative annual increase was estimated as 2.1% (1.4%; 2.8%). Overall, there were no significant differences in trends among boys and girls (annual increase: 2.3% (1.3%; 3.2%) vs. 1.9% (0.9%; 2.9%), $p = 0.592$). Among boys, significant increasing trends were observed in the 0–4, 5–9 and 10–14 years age groups (2.3% (0.1%; 4.5%) vs. 2.9% (1.2%; 4.7%) vs. 2.3% (0.8%; 3.9%), $p < 0.05$ each), but no change in adolescents (0.6% (-2.2%; 3.4%), $p = 0.529$). Among girls, significant increasing trends were observed in the 5–9 and 10–14 years age groups (3.7% (2.0%; 5.4%) vs. 2.9% (1.1%; 4.7%), $p < 0.05$ each), there was no change among 0–4 year-olds (0.1% (-2.1%; 2.4%), $p = 0.909$). The incidence among 15–19 year old girls tended to decrease (-2.6% (-5.3%; 0.1%), $p = 0.062$).

Conclusion: The data suggest that T1D incidence in childhood and adolescents is still increasing in Germany, unlike in Nordic European countries. However, age-specific trends are not uniform in both sexes. Based on the current data, there are annually about 3,200–3,700 children and adolescents newly diagnosed with T1D in Germany underlining the public health importance of the disease. Causes of the continuous rise and, in particular, the differential age-specific trends of T1D incidence between sexes have still to be identified.

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Insulin pumps or injections in children with type 1 diabetes

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Background and aims: During the last decades the percentage of children/adolescents with type 1 diabetes and insulin pump treatment (continuous subcutaneous insulin infusion [CSII]) increased substantially. In Germany about 40% of the children younger than 6 years are currently treated with

CSII. It was the aim of the trial to analyse the quality of diabetes control and patients' ability for a sufficient diabetes self-management using CSII versus injection therapy.

Materials and methods: All children/adolescents ($n=901$, age 11.5 ± 4.0 , diabetes duration 4.0 ± 3.6 years) with type 1 diabetes were enrolled in the trial, admitted to an in-patient rehabilitation during the period 04/2004–10/2010.

Results: At the time of hospital admission $n=707$ patients (78%) had an injection therapy (IT), $n=194$ (22%) had a CSII. Quality of diabetes control (IT vs CSII: HbA1c 8.72 ± 2.26 vs $8.35\pm 1.71\%$, $p=0.09$), mean amplitude of daily blood glucose excursions (9.4 ± 3.4 vs 9.8 ± 3.2 mmol/l, $p=0.22$), incidences of acute complications (hypo-/hyperglycaemia) were comparable. Children/Adolescents with CSII had a longer diabetes duration (5.3 ± 3.7 vs 3.6 ± 3.5 years, $p<0.01$), better postprandial blood glucose levels (10.0 ± 3.0 vs 12.2 ± 3.3 mmol/l, $p=0.03$) and a higher frequency of blood-glucose self-tests (45.4 ± 13.3 vs 38.2 ± 11.5 self-tests/week, $p<0.01$). Neither there were substantial differences in the group of children younger than 6 years: CSII ($n=34$) vs IT ($n=58$) (HbA1c 7.44 ± 0.82 vs $7.45\pm 1.21\%$, $p=0.99$, mean amplitude of daily blood glucose excursions 10.5 ± 3.4 vs 10.4 ± 3.1 mmol/l, $p=0.87$, number of hypoglycaemia during the preceeding 4 weeks 15.4 ± 11.1 vs 14.1 ± 11.7 , $p=0.61$). However, CSII offers children and adolescents more flexibility, more effective diabetes self-management and in adolescents a higher quality of life ($p<0.01$).

Conclusion: In children/adolescents CSII is a highly effective therapy. It offers children and adolescents a good and to the injection therapy comparable quality of diabetes control (with even lower postprandial blood glucose levels), but a significant better flexibility, more effective diabetes self-management and additionally for adolescents a higher quality of life.

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The timing of transition from paediatric to adult care for childhood-onset type 1 diabetes in Japan: DERI mortality study

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Background and aims: The timing for transition of patients with childhood-onset type 1 diabetes (T1DM) from pediatric care (PC) to adult care (AC) has become a subject of much debate in recent years, which led to a position statement being released by the ADA transition working group, and this topic is also being hotly debated in Japan, where not a few patients appear to continue to seek PC even after attaining adulthood. The aim of this study is to follow up patients with childhood-onset T1DM for a maximum duration of 40 years to see how they transitioned from PC to AC and to examine the factors that contributed to their transition.

Materials and methods: The study participants consisted of 1,299 patients (525 males/774 females) from two nationwide T1DM surveys in Japan who had been diagnosed as having T1DM at less than 15 years of age between 1965 and 1979. Their attending physicians and affiliations were determined by questionnaire surveys conducted every 5 years. Patients were classified as having received either PC or AC when they were 15 years old. In addition, for those who sought PC at that time, the timing for transition from PC to AC as well as factors such as their age at diagnosis, sex, their physicians' board certification status, and calendar year of diagnosis that contributed to their transition were assessed by Kaplan-Meier and Cox proportional hazard analyses as of 1 January 2010. All statistical analyses were performed by using SAS version 9.3.

Results: At the age of 15, 44.0% of the participants ($n=571$) and 55.4% ($n=720$) consulted PC (PC group) and AC (AC group), respectively. Their mean age at diagnosis according to their attending physicians was 7.9 ± 3.7 vs. 8.6 ± 3.7 years, respectively (PC group vs. AC group; $P<0.0001$). There were no significant sex differences between the PC and AC groups (PC, 46.7% vs. 42.4%; AC, 53.1% vs. 57.6%; $P=0.11$). Of the 571 patients in the PC group, 74.8% ($n=569$), 58.1% ($n=562$), 45.1% ($n=483$), and 15.1% ($n=394$) sought PC at 20, 30, 40, and 50 years of age, respectively. Cox proportional hazard analyses for patients with childhood-onset T1DM indicated that the more recent the calendar year of their diagnosis, the younger their age at diagnosis, and the more likely they were to continue to consult diabetes specialists, the significantly higher the risk of continuing to seek PC after attaining adulthood (hazard ratios, 1.03, 0.93, 1.80; $P=0.0046$, <0.0001 , <0.0001), while this risk was not significantly different between the sexes.

Conclusion: A majority of patients with childhood-onset T1DM sought AC at the age of 15 years in Japan. However, more than half of those who sought PC at the age of 15 continued to seek PC even after the age of 30 years, suggesting that their transition from PC to AC was far from smooth. Further study is needed to identify the obstacles to their smooth transition to AC.

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Predictor factors associated with higher risk of developing disordered eating behaviour in a group of female adolescents with type 1 diabetes mellitus

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Background and aims: Patients with type 1 Diabetes Mellitus (T1DM) are at high risk for disordered eating behavior (DEB), due to an emphasis on nutrition management in diabetes care. In particular females with T1DM may be principally vulnerable to develop DEBs due to factors related to the onset and management of the disease. Moreover DEBs can have negative effects on the management of T1DM. Because DEBs could be dangerous for people with T1DM, it is important to identify not only clinical and sub-clinical factors but also predictors of DEBs in adolescent females with T1DM. The aims of this study were: 1) to examine disordered eating attitudes and behaviors and body dissatisfaction in a sample of Italian adolescent and young adults with T1D; 2) to analyze what factors before and at the onset of T1DM could be associated with an increased risk of developing DEB.

Materials and methods: We enrolled 44 patients, adolescent females, aged 12–20 years old, in therapy with multiple daily insulin injections (MDI), recruited from our center. Inclusion criteria were: T1DM, duration of diabetes for at least 12 months, daily insulin dose of at least 0.5 U/Kg; age at the onset of T1DM 9–16 years, Caucasian race; no history of diabetes complications or autoimmune disorders. Patients with previously diagnosed eating disorders were excluded. Anthropometrical characteristics (weight, height, BMI, BMI z-score) were collected before and at the onset. The bio-clinical data at the T1DM onset (age, HbA1c, blood pH, weight loss) and the family data (including mother's and father's BMI, socio-economic status) were also collected. All patients completed the Eating Disorder Examination Questionnaire (EDE-Q); it assesses the frequency of key behavior such as binge eating, objective or subjective episodes of overeating, self-induced vomiting, laxative and diuretics misuse, and the other feature of eating disorder. A patient with an EDE-Q score >1 SD from the mean was considered pathological and with a high risk of developing eating disorder.

Results: 44 patients completed the EDE-Q test, 6 of them reported a score >1 SD (6/44, 13.2%) and were considered at risk of developing eating disorders. The logistic regression showed that, among all the anthropometric and metabolic variables, the only one that could be considered a predictor of a high score at the EDE-Q test (>1 SD from the mean) was the BMI z-score before the onset of diabetes. The BMI z-score (index of overweight/obesity) before the onset of T1DM was then identified as an independent predictor of a positive EDE-Q test ($p<0.05$, OR=2.029, 95% CI 1.059–3.889).

Conclusion: The presence of overweight/obesity, represented by BMI z-score, before the onset of T1DM could be considered an independent predictor of an increased risk of developing eating disorders in the medium/long term. Therefore, the overweight/obese girls with T1DM and their families should be included in adequate prevention programs for the management of DEBs since the onset of diabetes and should be followed by a multidisciplinary diabetes team with a peculiar attention to nutrition and psychological management.

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International comparison of quality of life in children and adolescents with type 1 diabetesA. Lukács¹, E. Kalyva², M. Abdul-Rasoul³, L. Barkai^{1,4},¹Faculty of Health Care, Department of Theoretical Health Sciences, University of Miskolc, Hungary, ²The International Faculty of the University of Sheffield, City College, Thessaloniki, Greece, ³Faculty of Medicine, Kuwait University, Kuwait, ⁴Postgraduate Institute of Pediatrics, Medical and Health Science Center, University of Debrecen, Miskolc, Hungary.

Background and aims: The ultimate goal of chronic patient care is to maintain or improve the quality of life. The patients' quality of life may be affected the successful disease management in short- and long term. There is a lack of researches examining the quality of life in pediatric diabetic population across countries, because of the lack of appropriate validated age- and disease-specific quality of life instruments. Our research aimed to assess the diabetes-specific quality of life in children and adolescents with type 1 diabetes from different geographically located countries using the child self- and parent proxy-reports. We looked for the relationship between the glycemic control and quality of life.

Materials and methods: A total of 416 youths with type 1 diabetes (212 boys and 204 girls) aged 8–18 years participated in the study (84 Greek, 135 Hungarian and 197 Kuwait participants). There were no significant differences among the study participants in gender, age and glycemic control. Diabetes-specific quality of life was measured using the Pediatric Quality of Life™ 3.0 Diabetes Module culturally adapted in every country. Glycemic control was expressed by HbA1c. Data were analysed with Chi-square test, paired t-test, one-way analysis of variance. The difference across countries was evaluated using Tukey post hoc test. SPSS 19.0 was used for statistical analysis.

Results: There were significant differences among countries both in self- ($F(2, 413)=13.17$, $p<0.001$) and proxy-report ($F(2, 413)=30.02$, $p<0.001$). Greek youths ($M=62.18$, 95% CI [59.07, 65.29]) had significantly lower quality of life scores than the Hungarian ($M=71.32$, 95% CI [69.25, 73.40], $p<0.001$) and Kuwait youths ($M=68.35$, 95% CI [66.60, 70.10], $p=0.001$). These results were confirmed by the proxy reports. Parents of Greek youths ($M=55.77$, 95% CI [52.91, 58.62]) reported significantly lower quality of life scores than parents of Hungarian ($M=67.80$, 95% CI [65.80, 69.79], $p<0.001$) and Kuwait youths ($M=59.75$, 95% CI [58.09, 61.40], $p=0.031$). Comparisons between quality of life of Hungarian and Kuwait youths we found statistically significant differences only in parents' report ($p<0.001$). We found negative significant correlation between the glycemic control and quality of life for the overall sample ($r(416)=-0.212$, $p<0.001$) and for each country (Gr: $r(84)=-0.384$, $p<0.001$, Hu: $r(135)=-0.207$, $p=0.016$, Ku: $r(197)=-0.207$, $p=0.004$). It was the case in the parent proxy-report (Gr: $r(84)=-0.387$, $p<0.001$; Hu: $r(135)=-0.170$, $p=0.049$) except Kuwait ($r(197)=-0.067$, $p=0.349$).

Conclusion: Regardless the cultural and geographical differences, relationship is found between glycemic control and diabetes-specific quality of life. Parents perceived their children's quality of life worse than children themselves. Despite the similar clinical status of the study participants, the perceived quality of life of diabetic youths differed across cultures. There is a challenge to find ways of improving the quality of life of youths and maintain throughout childhood and adolescence in every culture.

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Effect of aerobic exercise on HDL function in women with polycystic ovary syndrome: a randomised controlled trial

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Background and aims: Polycystic ovary syndrome (PCOS) is associated with increased cardiovascular risk. Insulin resistance and low levels of high-density lipoprotein (HDL) cholesterol are considered as potential mechanism for the increased cardiovascular risk. Here we determined whether HDL function in PCOS women is different from healthy women who are more insulin sensitive. Since aerobic exercise (AE) enhances insulin sensitivity and is an effective treatment option in PCOS women we determined whether PCOS women improve their HDL concentrations and HDL function in response to 12 weeks of AE.

Materials and methods: HDL function, assessed by cholesterol efflux capacity, paraoxonase-1 (PON1) activity and total anti-oxidative capacity was compared between sedentary women with PCOS ($n=25$) and age- and sex-matched non-PCOS, women ($n=12$). PCOS women were then randomized to a 12-week intervention of AE ($n=12$), or no treatment ($n=13$) to analyze the effect of AE on fasting serum insulin levels and glucose tolerance to a meal, and on HDL function. AE consisted of 5 supervised exercise sessions per week on a stationary bicycle to elicit heart rates corresponding to 65% of participants' pre-training VO2max. Participants in the control group maintained their regular sedentary behavior.

Results: The HDL of PCOS women exhibited impaired cholesterol efflux capacity compared to insulin-sensitive healthy women ($15.9\% \pm 2.2$ versus $17.8\% \pm 2.4$, $p=0.034$), whereas PON1 activity and total antioxidative function showed no differences. Although AE resulted in a significant increase in VO2 max ($+4.53$ ml/kgFFM/min ± 2.52 , $p=0.003$), and improved glucose tolerance, no effect on HDL concentration and function was observed.

Conclusion: PCOS women have an impaired cholesterol efflux capacity, which cannot be normalized by a 12 week AE despite improvement in glucose tolerance and endurance capacity.

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Association between bone metabolism and resting energy expenditure in postmenopausal women with type 2 diabetes

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Background and aims: Osteoporosis negatively affects quality of life in individuals with diabetes. While bone fractures occur more easily in patients with both type 1 diabetes (T1D) and T2D compared to non-diabetics, the pathology of osteoporosis is different between T1D and T2D. Although bone mineral density (BMD) is increased in patients with poorly controlled T2D, the bone quality is low. It is therefore important to determine criteria for osteoporosis other than BMD in patients with T2D. Recently, it has been reported that bone resorption suppression may prevent T2D, and BMD correlates more strongly with basal metabolic rate than body mass index (BMI) in African Americans. These studies imply a direct association between bone metabolism and basal metabolic rate. The aim of this study was to examine the relationship between bone metabolism and resting energy expenditure (REE) in postmenopausal women with T2D.

Materials and methods: Postmenopausal Japanese women with T2D were recruited from the outpatient clinic at the Diabetes Center of our University. 46 patients with over 1-year attendance, no other diseases except hypertension, dyslipidemia and overweight ($BMI \leq 30$ kg/m²), no history of taking dietary supplements for previous 3 months, were included in this study. Fasting serum procollagen type 1 N-terminal propeptide (P1PN), Carboxy-terminal collagen crosslink's-1 (CTX-1), intact parathyroid hormone, 25-hydroxyvitamin D (25[OH]D), urine microalbumin, body composition and REE were evaluated. BMD was examined using dual-energy X-ray absorptiometry of the nondominant distal radius. Data are presented as mean \pm standard deviation.

Results: The mean REE of the patients (940 ± 156 kcal) was lower than the predicted value (1177 ± 98.3 kcal). The mean T-score was low with high variance (1.7 ± 1.6). 18 patients (39%) met the criterion for osteoporosis. Although 12 patients (26%) had a history of bone fractures, there was no difference in REE or BMD between the patients with or without the history of fractures. The respiratory quotient (0.87 ± 0.01) was positively correlated with serum 25(OH)D (21.5 ± 7.4 ng/mL). REE was positively correlated with BMI (24.5 ± 3.6 kg/m²), serum calcium (9.2 ± 0.3 mg/dL), HbA_{1c} for the prior 6 months ($7.6\% \pm 1.1\%$), and the ratio of serum P1PN to CTX-1 (37.5 ± 11.7 μ g/L : 0.36 ± 0.1 ng/mL).

Conclusion: The present study shows that bone metabolism was significantly associated with REE ($p<0.009$). The basal metabolic rate interrelated with bone turnover in postmenopausal women with T2D. Declining bone turnover related to low REE may result in decreased therapeutic efficacy of bone resorption suppression.

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Comparison of the effect of minodronic acid hydrate and bazedoxifene on biochemical markers of bone turnover in postmenopausal type 2 diabetes women with low bone mass

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Background and aims: Although patients with type 2 diabetes (T2D) are at significant risk for well-recognized diabetic complications, including macrovascular disease, retinopathy, nephropathy, and neuropathy, it is also clear that T2D patients are at increased risk for fragility fractures. It is suggested abnormalities in not only bone material strength but also bone microarchitecture (bone quality) are risk factors of bone fractures in T2D. It is reported that urinary pentosidine levels predict the future fracture independent of bone turnover and bone mineral density (BMD) without treatment for osteoporosis. Pentosidine is a surrogate marker of whole advanced glycation end products, which is characterized as non-enzymatic collagen cross-linking. Non-enzymatic cross-links reduce the mechanical and biological integrity of bone and so pentosidine is one of bone quality markers. It has been reported that selective estrogen receptor modulator (SERM) decreases urinary pentosidine and ameliorates bone quality in postmenopausal women with osteoporosis. However, effects of osteoporosis medication to bone quality have not been proven in T2D with osteoporosis. Thus, we administered bisphosphonate or SERM to postmenopausal T2D women with low bone mass and evaluated the effects of bone turnover including bone quality markers.

Materials and methods: We measured femoral neck (FN) and lumbar spine (LS) BMD of postmenopausal women with T2D by dual-energy X-ray absorptiometry and 31 patients with a T score ≤ -2.0 at the FN or LS in this test were randomized 1:1 to receive minodronic acid hydrate ($n=15$) or bazedoxifene ($n=16$). Changes in bone turnover and quality markers, BMD at the FN and LS, and common clinical data involved in T2D were assessed at 6 months. This was a prospective observational study. Primary endpoint was set as the reduction of urinary pentosidine level. A total of 31 patients consented to participating in this study. On average, the patient group was aged 69 ± 7 years and they had been living with T2D for the past 15.5 ± 10.7 years. Their HbA1c was $7.0 \pm 0.7\%$ and BMI was 24.0 ± 3.8 .

Results: There was no difference in urinary pentosidine levels between the groups. No significant reduction was seen in both groups, which urinary pentosidine levels were very high and more than the level considered as a risk level of future fractures. In the other bone turnover and quality markers, minodronic acid hydrate significantly decreased serum P1NP levels, urinary DPD and serum ucOC compared with bazedoxifene (-58.6% versus -19.1% , -30.1% versus 1.6% , -55.3% versus -29.1% , respectively). Significantly greater increases in BMD were observed with minodronic acid hydrate treatment at the FN or LS (6-mo treatment difference: 2.3% , FN; 2.4% , LS; $p < 0.05$) but no changes were observed with bazedoxifene therapy. Bazedoxifene significantly decreased serum non HDL-C levels compared with minodronic acid hydrate (-13.6 mg/dl versus 5.4 mg/dl; $p < 0.05$). Both treatments didn't aggravate diabetes conditions.

Conclusion: Urinary pentosidine levels did not change in both treatments in this study. Minodronic acid hydrate showed significantly improvement in BMD and bone turnover markers compared with bazedoxifene therapy in also T2D patients. Bazedoxifene may ameliorate lipid metabolism in T2D women with osteoporosis.

Clinical Trial Registration Number: UMIN000008783

PS 114 Clinical observations in type 2 diabetes

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Clinical features and management of new-onset diabetes mellitus presenting with diabetic ketoacidosis in China: a multicentre, clinic-based study

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Background and aims: Diabetic ketoacidosis (DKA) is a serious acute complication of diabetes, resulting from severe insulin deficiency and an excess of counter-regulatory hormones. Under certain circumstance, new-onset diabetes patients may present with DKA, which is typical for type 1 diabetes mellitus (T1DM) or ketosis-prone diabetes in western countries. However, there is little information on hospitalization for this kind of diabetes patients in the Chinese population. The present study is to evaluate the clinical features and management of new-onset diabetes mellitus presenting with DKA in China.

Materials and methods: A retrospective cohort study of adult and adolescents new-onset diabetes patients hospitalized with DKA between 2010 and 2012 were carried out in fifteen tertiary hospitals around China. Clinical and laboratory data were collected. Patients were classified based on clinical features. Groups were compared for differences in vital statistics and biochemical profiles at presentation.

Results: The study cohort comprised 255 new-onset diabetes patients presenting with DKA: 115 patients (45.1%) with type 2 diabetes mellitus (T2DM), 98 patients (38.4%) with T1DM, 24 patients (9.4%) with atypical DM (ADM) and 18 patients (7.1%) with Fulminant T1DM. T1DM exceeded T2DM in the age group less than 40 years while T2DM predominated in the age group more than 40 years. Male patients were predominant in T1DM, T2DM and ADM group. The most common precipitating factors were unknown reason (36.9%) and infection (29.0%). The typical symptoms included polyuria/polydipsia (72.9%), nausea/vomiting (67.1%), dehydration (27.1%), variable degree of confusion (26.7%) and abdominal pain (22.4%). More gastrointestinal symptoms and dehydration were found in T1DM due to severe ketoacidosis. The levels of blood glucose and HbA1c were 25.3 ± 11.1 mmol/L and $12.1 \pm 3.1\%$ at admission. About 89.0%, 74.4% and 55.9% of patients were evaluated for HbA1c, beta-cell function and autoantibodies for classification. The complications included electrolyte disturbance (71.4%), arrhythmia (19.6%), hyperosmotic state (14.5%), renal failure (6.7%), shock (3.1%), heart failure (0.8%) and cerebral edema (0.8%). During the first day after admission, total fluid supplement were 3550.4 ± 1904.1 ml. Low doses insulin infusion lasted for 2.8 ± 5.4 days. The disappearance of urine ketone body took 3.3 ± 2.7 days. Therapeutic regimen was markedly different within the four groups when they discharged from hospitals.

Conclusion: New-onset diabetes patients presenting with DKA occur in a spectrum of diabetes types in China, including T2DM, T1DM, ADM and Fulminant T1DM. Its clinical heterogeneity has significant implications for classification and management of diabetes.

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Effects of cardiovascular risk factors in urban community in patients with type 2 diabetes mellitus: a 48-months prospective trial

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Background and aims: To assess whether prospective interventions have long-term effects on the risk of diabetes-related macrovascular complications in patients with type 2 diabetes mellitus in urban communities of Beijing.

Materials and methods: A total of 3,264 type 2 diabetic subjects (aged 20–80 years) was recruited from 15 community health centers in Beijing in 2008.

The subjects were divided into three groups: DM group (n=930), HTN group (n=1397), and CVD group (n=937). By using Framingham risk score (FRS), the subjects in afore three groups are subdivided into risk categories of 20% (high Framingham risk strata). After 48 months, study participants were followed-up to assess the long-term effects of the interventions.

Results: Subjects with CVD in diabetes were more prone to be older, have a longer duration of diabetes, higher systolic blood pressure and diastolic blood pressure than that of DM group ($P<0.01$, respectively). The mean NC at the baseline was significantly higher in HTN and CVD group than that in DM group ($P<0.05$). In the post-intervention, participants in CVD group or HTN group comparing the DM group, higher levels were detected on blood pressure, and lipid profiles ($P<0.01$, respectively). In 48-months of follow-up, no significant increase of FRS could be demonstrated in low framingham risk strata and median framingham risk strata when compared with baseline levels. In particular, a significant reduction of FRS was found in the high framingham risk strata at the end of follow-up. In COX multivariate analyses, participants in the HTN group and CVD group had a higher incidence of events than those in the DM group (HR 2.561; 95% CI 1.043–6.289; HR 4.678; 95% CI 1.937–11.298).

Conclusion: This study demonstrated for the first time the existing relationship between NC and CVD in diabetic patients. Multi-factorial intervention of CVD risk factors in community for 48-months results in favorable changes in cardiovascular risk factors, and lowered the estimated 10-year risk for CVD events. Multi-factorial intervention of CVD risk factors in community is important to prevent the incidence of events in hypertension or CVD in Chinese diabetic patients.

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Cardiovascular risk factors outcomes in patients with newly-diagnosed type 2 diabetes. The Basque country 10-yr prospective diabetes study

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Background and aims: To describe the cardiovascular risk factor outcomes in newly-diagnosed type 2 diabetes in the Basque Country Prospective Complications and Mortality Study.

Materials and methods: A 10-year prospective population-based cohort study was performed with 777 newly-diagnosed type 2 diabetic patients older than 24 years in a Sentinel Practice Network. We recorded cardiovascular risk factors at baseline and during the 10-year follow up study. High blood pressure (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 80 mmHg), elevated LDL ≥ 2.58 mmol/l, low HDL < 1.29 mmol/l in women and < 1.03 mmol/l in men, hypertriglyceridemia (triacylglycerol ≥ 1.69 mmol/l), HbA_{1c} $\geq 7\%$ (≥ 53 mmol/mol), and albuminuria (albumin/creatinine ratio ≥ 3 mg/mmol) were recorded. Obesity (BMI ≥ 30 kg/m²), and adiposity (% body fat) were calculated. The air displacement plethysmography prediction equation was used to measure adiposity (obesity: above 25.1% and 35.1% in males and females, respectively). The patients were classified as sedentary if they rarely exercised and active if they performed three or more hours of exercise per week. With regard to smoking habits, the patients were classified as non-smokers, and smokers.

Results: Baseline characteristic of the patients are shown in table. At the end of the study, all cardiovascular risk factors outcomes were favorable, improving the percentage of patients at target for blood pressure from 87% to 78.3%, and LDL from 88.8% to 65%. However, the patients at HbA_{1c} $\geq 7\%$ increased from 29% to 35% in the cohort study. Sedentary life style and obesity did not change over time. Smoking habits decreased from 16.1% to 8.3%. Treatment with statins, blood pressure drugs and anti-hyperglycemic drugs increased in the follow-up study.

Conclusion: Cardiovascular risk factors prevalence and percentage of patients upper control target are very high. All risk factors except HbA_{1c}, improved or maintained in newly-diagnosed type 2 diabetic patients during 10-yr. follow-up study.

	Men n=410	Women n=367	Total n=777	p value
Age at diagnosis (mean \pm SEM)	61.3 \pm 0.5	65.9 \pm 0.6	63.5 \pm 0.4	<0.0001
Smoking (%)	28.1	3.3	16.1	<0.0001
Obesity (IMC ≥ 30) (%)	35.0	55.6	44.7	<0.0001
Adiposity (%)	91.0	96.9	93.7	<0.001
Sedentary (%)	30.2	47.6	38.3	<0.0001
Mean \pm SEM	Mean \pm SEM	Mean \pm SEM		
Total cholesterol (mmol/l)	5.58 \pm 0.06	5.80 \pm 0.05	5.68 \pm 0.04	<0.01
LDL (mmol/l)	3.56 \pm 0.05	3.68 \pm 0.05	3.62 \pm 0.03	0.080
HDL (mmol/l)	1.28 \pm 0.02	1.44 \pm 0.02	1.36 \pm 0.01	<0.001
Triacylglycerol (mmol/l)	1.79 \pm 0.07	1.57 \pm 0.04	1.69 \pm 0.04	<0.01
HbA _{1c} % (mmol/mol)	6.6 (49) \pm 0.1	6.7 (50) \pm 0.1	6.6 (49) \pm 0.1	0.312
Systolic BP (mmHg)	136.4 \pm 0.8	140.4 \pm 0.8	138.3 \pm 0.6	<0.001
Diastolic BP (mmHg)	80.7 \pm 0.5	81.7 \pm 0.5	81.2 \pm 0.3	0.136
Risks factors (upper control target)	%	%	%	
Cholesterol (≥ 5.17 mmol/l)	64.1	74.0	68.8	<0.01
Triacylglycerol (≥ 1.69 mmol/l)	38.6	34.6	36.7	0.261
LDL (≥ 2.58 mmol/l)	86.2	91.7	88.8	<0.05
HDL (< 1.03 mmol/l men and < 1.29 mmol/l women)	22.3	36.2	29.0	<0.0001
High blood pressure ($\geq 140/80$ mmHg)	76.8	81.5	79.1	0.110
HbA _{1c} $\geq 7\%$ (≥ 53 mmol/mol)	27.7	30.3	29.0	0.437
Atherogenic dyslipidemia ^a	13.0	23.0	17.7	<0.001

^aHDL < 1.29 mmol/l and triacylglycerol ≥ 1.69 mmol/l in women and HDL < 1.03 mmol/l and triacylglycerol ≥ 1.69 mmol/l in men.

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The relationship between coronary artery disease and peripheral artery disease in diabetes patients

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Background and aims: In step with the aging of the population and the increasing number of diabetes patients, patients with peripheral artery disease (PAD) are increasing in number along with those with coronary artery disease (CAD). It is important for PAD to be detected early and managed because it has a poor prognosis and higher mortality rate than malignant disease. This study examined the associations of CAD and PAD in patients with type 2 diabetes mellitus (DM).

Materials and methods: Subjects were 1368 ambulatory patients with type 2 diabetes (795 males, 573 females). They were classified into CAD+ and CAD- groups, or into PAD+ and PAD- groups. Those with ischemic findings such as abnormal Q, ST depression and negative T on resting electrocardiogram or a past history of angina pectoris or myocardial infarction were categorized as CAD+, otherwise as CAD-; those with ankle-brachial index (ABI) ≤ 0.99 or with a history of PAD were categorized as PAD+, otherwise as PAD-. They were further divided into two groups: one with diabetic retinopathy and the other without it. Diabetic nephropathy was treated as positive if urinary albumin excretion was 30 mg/gCr or larger and as negative if it was less than 30 mg/gCr. We compared risk factors between CAD which is representative of diabetic macrovascular disease and PAD.

Results: The characteristics of the 1368 patients with type 2 DM were as follows: age, 64.1 \pm 12.0 (SD) years; duration of DM, 11.4 \pm 10.0 years; BMI, 24.5 \pm 8.0 kg/m²; blood pressure (BP), 135 \pm 19/76 \pm 13 mmHg; and hemoglobin A_{1c}, 7.9 \pm 2.0 %. Three hundred thirty-two patients had retinopathy, while 795 did not. Nephropathy afflicted 572 patients but did not afflict 720 patients. The CAD+ group comprised 498 subjects (36.4%). After data correction, independent risk factors for CAD were age (OR1.03, 95%CI 1.01-1.04, $p<0.0005$), nephropathy (OR1.53, 95%CI 1.12-2.09, $p<0.01$), retinopathy (OR1.52, 95%CI 1.07-2.15, $p<0.05$) and smoking habit (OR1.58, 95%CI 1.09-2.29, $p<0.05$). The PAD+ group comprised 227 subjects (16.6%). After data correction, age (OR1.05, 95%CI 1.03-1.07, $p<0.0001$), waist diameter (OR1.03, 95%CI 1.01-1.05, $p<0.005$), nephropathy (OR1.60, 95%CI 1.06-2.42, $p<0.05$), retinopathy (OR2.61, 95%CI 1.67-4.08, $p<0.0001$), male gender (OR2.73, 95%CI 1.62-4.64, $p<0.0005$) and smoking habit (OR3.37, 95%CI 1.98-5.85, $p<0.0001$) were independent risk factors for PAD. The cut-off value of the ROC curve for age was 75 years (sensitivity:0.39, specificity:0.82). PAD was found in 27% of the CAD+ group ($p<0.0001$), while CAD was found in 59% of the PAD+ group ($p<0.0001$).

Conclusion: About 30% of CAD patients had PAD while about 60% of PAD patients had CAD. Factors common to CAD and PAD were age, smoking habit, nephropathy and retinopathy. Especially PAD is considered clinically worthy of note in people over 75 years old. In our increasingly aging country, we consider it essential to educate the public as well as diabetes patients about the importance of early diagnosis of PAD which carries a poor prognosis.

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Outcome reduction with an initial glargine intervention and legacy effects (ORIGINALE)

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Background and aims: ORIGIN was a 2 x 2 factorial randomised trial assessing cardiovascular and other effects of insulin glargine vs standard care and omega-3-fatty acids vs placebo among people with IFG, IGT or early type 2 diabetes and other cardiovascular risk factors. After a median 6.2-year follow-up, insulin glargine had a neutral effect on cardiovascular outcomes and cancers, and reduced the incidence of diabetes in people with IFG or IGT. Omega-3-fatty acids also had a neutral effect on cardiovascular outcomes. ORIGIN participants who consented to the ORIGIN and Legacy Effects Study (ORIGINALE) were followed for more than 2 years after the trial finished to determine the longer term effect of therapy on cardiovascular outcomes and new diabetes.

Materials and methods: By the end of March 2014, consenting participants completed up to 2 study visits to ascertain anthropometrics, blood pressure, creatinine, A1C, medication use and outcomes. Cumulative incidence, hazard ratios and odds ratios from the time of randomisation to the end of follow-up will be calculated for the ORIGIN trial outcomes. Participants will be analysed in the groups to which they were randomised.

Results: At the end of ORIGIN, 10,544 of the original 12,537 participants were alive and followed at 553 sites. 283 sites (comprising 6,497 participants) agreed to participate in ORIGINALE and obtained ethics approval. To date, at least 5721 participants from 274 sites have contributed data comprising approximately 96 new MIs, 63 new strokes, 194 new cardiovascular deaths and 231 new cancer outcomes. Verification of events and data accuracy are ongoing. Analyses comparing insulin glargine vs standard care, and omega-3-fatty acids vs placebo will be completed by August 2014.

Clinical Trial Registration Number: NCT00069784

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Effect of three insulin regimens on carotid intima-media thickness in patients with type 2 diabetes: the randomised Copenhagen Insulin and Metformin Therapy (CIMIT) trial

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Background and aims: Post prandial hyperglycaemia may be associated with increased risk of cardiovascular disease in patients with type 2 diabetes. Insulin analogue regimens targeting post prandial and/or fasting plasma glucose may therefore influence the risk of cardiovascular disease differently. Carotid intima-media thickness (IMT) is a frequently used risk marker for cardiovascular disease. The aim of the Copenhagen Insulin and Metformin Therapy (CIMIT) trial was to evaluate the effects of three insulin analogue regimens in combination with metformin or placebo. The metformin/placebo comparison has previously been reported. This abstract reports the insulin regimen comparisons.

Materials and methods: The CIMIT trial is an investigator initiated, 3 x 2 factorial, treat-to-target ($HbA_{1c} \leq 7.0\%$ (53 mmol/mol)), multicenter randomised clinical trial. 412 participants with type 2 diabetes, $HbA_{1c} \geq 7.5\%$

(58 mmol/mol), receiving oral antidiabetic agents for at least one year and/or insulin for at least three months, were randomised 1:1:1 to 18 months open label treatment with one of three insulin regimens: insulin aspart biphasic one to three times daily (n=137) versus insulin aspart three times daily in combination with insulin detemir once daily (basal-bolus, n=138) versus insulin detemir once daily (n=137). Primary outcome measure was change in mean carotid IMT. Other outcomes were change in HbA_{1c} , weight, and insulin dose, and risk of hypoglycaemia and serious adverse events. After multiple imputations of missing data on the primary outcome, intention-to-treat analyses adjusting for baseline and stratification variables were performed.

Results: 90% (biphasic group), 80% (aspart+detemir group), and 72% (detemir group) of the participants completed the trial. Mean carotid IMT changed by -0.009 mm (95% confidence interval -0.022 to 0.004, $P=0.17$) in the biphasic group, 0.000 mm (-0.013 to 0.013, $P=0.99$) in the aspart+detemir group, and -0.012 mm (-0.025 to 0.000, $P=0.06$) in the detemir group. These changes were not significantly different between the groups. HbA_{1c} was significantly more reduced ($P<0.001$) in the aspart biphasic group (-1.0% (-1.2 to -0.8) (-11 mmol/mol (-13 to -9)) compared with the aspart+detemir (-0.4% (-0.6 to -0.3) (-4 mmol/mol (-7 to -3)) and the detemir (-0.3% (-0.4 to -0.1) (-3 mmol/mol (-4 to -1)) groups. Weight gain was significantly higher ($P<0.01$) in the biphasic group (3.3kg (2.7 to 4.0)) and the aspart+detemir (3.2kg (2.6 to 3.9)) groups compared with the detemir group (1.9kg (1.3 to 2.6)). Total insulin dose at end-of-trial was significantly higher ($P<0.001$) in the detemir group (1.6 IU/kg/d (1.4 to 1.8)) compared with the biphasic (1.0 IU/kg/day (0.9 to 1.1) and aspart+detemir (1.1 IU/kg/day (1.0 to 1.3)) groups. The number of participants with severe hypoglycaemia and other serious adverse events did not differ between the groups.

Conclusion: Despite major differences in HbA_{1c} , weight gain and insulin dose, no significant differences on the progression of carotid IMT were found during 18 months treatment with three frequently used insulin analogue regimens targeting post prandial and/or fasting plasma glucose in patients with type 2 diabetes. Further long-term trials are needed examining whether different insulin regimens affect cardiovascular risk differently.

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Glycemic variability and glucose control in post-transplant diabetes mellitus after renal transplantation

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Background and aims: Post-transplant diabetes mellitus (PTDM) is a common and serious complication after renal transplantation leading to increased cardiovascular morbidity and mortality. In patients with type 2 DM increased glycemic variability and poor glucose control have been associated with cardiovascular complications. We therefore aimed at determining glycemic variability and glucose control in patients with PTDM in comparison to type 2 DM.

Materials and methods: We studied 10 non-diabetic transplanted subjects (TR_ND), 10 transplanted subjects with PTDM (TR_D), and 9 non-transplanted type 2 diabetic subjects (T2_D) using Continuous Glucose Monitoring (CGM). Subjects had similar age (60.4±2.9, 57.6±2.2, 56.2±2.5 years, $P>0.26$) and BMI (26.9±1.3, 28.9±1.3, 29.6±2.1 kg/m², $P>0.24$). Subjects underwent CGM for 2 to 8 days. The following indices were computed: (i) Basic indices: glucose mean and standard deviation (SD); (ii) Indices of glycemic variability: $CONGA_n$ equal to the SD of the difference between values obtained 5 minutes apart; J-INDEX, equal to $0.001 \times (\text{mean} + \text{SD})^2$; MAGE equal to the arithmetic mean of the glycemic excursions greater than one SD; Lability Index equal to the mean of $(G_{n+1} - G_n)^2 / (h_{n+1} - h_n)$, where G_n is the n-th glucose value and h_n is the time when that value was collected; (iii) Indices of glucose control quality: GRADE equal to $425 \times [\log [\log(G_n)] + 0.16]^2$; Hyperglycaemia index, equal to the weighted average of hyperglycaemic values, for glucose values higher than 140 mg/dl; Hypoglycaemia index, for glucose values below 80 mg/dl; IGC equal to the sum of Hyperglycaemia and Hypoglycaemia indices.

Results: Mean glucose in TR_D and T2_D was higher than in TR_ND ($P<0.021$), whereas SD was different only between TR_ND and T2_D ($P<0.011$). No difference in Mean and SD was found between TR_D and T2_D ($P>0.3$). Among the variability indices, main differences among groups

were shown by J-INDEX (lower in TR_ND than in TR_D and in T2_D, $P<0.019$) and by Lability Index (lower in TR_ND and in TR_D than in T2_D, $P<0.029$). Among the control quality indices, GRADE was found significantly lower in TR_ND than in TR_D and in T2_D ($P<0.034$).

Conclusion: TR_D patients display worse glucose control than TR_ND, however, the degree of impairment parallels that of T2_D. Glycemic variability expressed by Lability Index is significantly higher in T2_D compared to TR_ND but also to TR_D. These data underscore potential important pathophysiological differences between type 2 DM and PTDM indicating that although development of PTDM leads to increased glycemic variability, the degree of variability is significantly higher in type 2 DM and may thus play a less important role in PTDM.

Table 1 – Values of the CGM indices in the three groups of subjects. Data are mean \pm SE

	TR_ND	TR_D	T2_D
Basic indices			
Mean (mg/dl)	107.4 \pm 3.7 ^{a,b}	126.7 \pm 7.6	131.7 \pm 4.6
SD (mg/dl)	22.0 \pm 2.2 ^b	28.1 \pm 2.7	31.2 \pm 1.9
Indices of glycemic variability			
CONGA _e (mg/dl)	2.51 \pm 0.37	2.39 \pm 0.20	3.05 \pm 0.30
J-INDEX (10 ⁻³ (mg/dl) ²)	17.0 \pm 1.4 ^{a,b}	24.7 \pm 3.2	26.7 \pm 1.4
MAGE (mg/dl)	37.7 \pm 3.7 ^b	51.0 \pm 6.6	56.9 \pm 4.1
Lability Index ((mmol/l) ² /hr)	0.175 \pm 0.029 ^b	0.219 \pm 0.037 ^c	0.364 \pm 0.064
Indices of glucose control quality			
GRADE (unitless)	2.17 \pm 0.33 ^{a,b}	4.14 \pm 0.92	4.69 \pm 0.44
Hyperglycaemia index (unitless)	5.17 \pm 2.06	4.48 \pm 1.66	3.23 \pm 0.90
Hypoglycaemia index (unitless)	0.81 \pm 0.13	1.27 \pm 0.29	1.30 \pm 0.06
IGC (unitless)	5.35 \pm 2.33	5.23 \pm 1.72	4.55 \pm 0.94

$P<0.05$ between a: TR_ND, TR_D; b: TR_ND, T2_D; c: TR_D, T2_D

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The association between metformin use and change of serum creatinine after administration of contrast medium

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Background and aims: Metformin is widely used in the treatment of type 2 diabetes mellitus. Because of a association between iodinated contrast medium and the development of lactic acidosis in patients taking metformin, it usually restricted the use of metformin in situations considered to be high risk for the development of lactic acidosis. Several guidelines recommend stopping metformin before injection of contrast medium. Nevertheless, this has been controversial, because cases of lactic acidosis in patient taking metformin occurred in patient who had high risk factors for lactic acidosis. Thus, we evaluated the change of serum creatinine and total CO₂ concentration in patients receiving metformin after administration of contrast medium.

Materials and methods: From January 2012 to August 2012, the patient's records after administration of contrast medium for patients receiving metformin were retrospectively reviewed. Patients were excluded if 1)cancer with chemotherapy or radiation therapy, 2)death after admission, 3)serum creatinine ≥ 3.0 mg/dL, or hemodialysis, 4) shock. Ninety patients were included in the final analysis. Serum creatinine is measured 1-3 days after administration of contrast medium. We measure serum total CO₂ content instead of measuring serum bicarbonate. The total CO₂ content includes the serum bicarbonate as well as available forms of carbon dioxide (such as dissolved CO₂ and carbonic acid). Generally, the serum bicarbonate comprises about 95% of the total CO₂ content, thus we can use this measurement as an excellent estimator of serum bicarbonate. We defined metabolic acidosis when total CO₂ level is below 23 mEq/L. The Wilcoxon's signed rank test was used to analyze the effects of contrast medium use on change in serum creatinine and total CO₂ content level from baseline to 1-3 days after contrast medium.

Results: The mean subject age was 67.4 \pm 10.5 years, and the mean A1C and metformin dosage of subjects were 7.3 \pm 1.6 kg/m² and 500(500-1000), respectively. Serum BUN level before and after administration of contrast medium were 17.2 \pm 9.2 and 15.0 \pm 7.9 mg/dL, respectively. Serum creatinine level before and after administration of contrast medium were 0.91 \pm 0.38 and 0.89 \pm 0.36 mg/dL, respectively. There was no significant change of BUN or Cr level after administration of contrast medium ($p > 0.05$). The total CO₂ level before and after administration of contrast medium were 24.3 \pm 3.5 mEq/L and 27.7 \pm 3.8 mEq/L, respectively. There was also no significant change of CO₂ level ($p = 0.435$). There was no significant relationship between increased creatinine and presence of metabolic acidosis (Table 1).

Conclusion: There is no significant increase or change of serum creatinine in patients with low risk of lactic acidosis after contrast media exposure. There-

fore, it imply that there is no evidence to stop metformin before administration of contrast medium in patients with normal renal function with low risk of lactic acidosis.

Increased serum creatinine	Presence of metabolic acidosis		P value
	Absent	Present	
Absent	42 (46.7%)	12(13.3%)	0.76
Present	27 (30.0%)	9 (10.0%)	

PS 115 Liver

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Non-invasive predictor of non-alcoholic fatty liver disease in Japanese patients with type 2 diabetes mellitus

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Background and aims: Individuals with type 2 diabetes mellitus (T2DM) have a higher risk of developing nonalcoholic fatty liver disease (NAFLD) than those without. The serum cytokeratin-18 fragment (CK-18) level has been suggested to be a biomarker of NAFLD, although its usefulness in patients with T2DM is unknown. The objective of the present study was to assess the usefulness of the serum CK-18 level as a biomarker for NAFLD in T2DM patients.

Materials and methods: The study was divided into two parts. In the first cross-sectional study, a total of 200 patients with T2DM and 58 healthy control subjects were recruited. NAFLD was diagnosed using ultrasonography, and the T2DM patients and nondiabetic control subjects were subdivided into groups with or without NAFLD (DM/NAFLD, DM/nonNAFLD, nonDM/NAFLD and nonDM/nonNAFLD, respectively). We used the scoring system, based on the criteria of hepatorenal echo contrast, vascular blurring and liver brightness and deep attenuation, to evaluate the presence of NAFLD. The ultrasonography scores ranged from 0 to 6 points, and a diagnosis of NAFLD was made for scores of ≥ 2 . In the subsequent longitudinal study, we evaluated the three-month change (Δ) in the CK-18 level and other parameters in 40 T2DM patients with NAFLD. Serum CK-18 levels were measured using ELISA.

Results: The median [IQR] level of serum CK18 was 158.4U/L [107.1–291.9] in the DM/NAFLD group, 96.1 U/L [74.1–142.6] in the DM/nonNAFLD group, 172.4 U/L [130.4–278.8] in the nonDM/NAFLD group and 120.4 U/L [97.5–158.1] in the nonDM/nonNAFLD group. The serum CK18 values were significantly higher in the NAFLD group than in the nonNAFLD group among both diabetic ($p < 0.0001$) and nondiabetic subjects ($p = 0.004$). The median [IQR] level of CK18 was not significantly different between the patients with diabetes and the controls (139.9 U/L [84.0–223.6] vs 147.4 U/L [104.0–198.7], $p = 0.63$). The CK-18 level was found to be an independent determinant of NAFLD (Odds ratio 1.01, 95%CI 1.00–1.02, $p = 0.004$) and was positively correlated with the ultrasonography score and AST and ALT levels in the T2DM patients. Positive correlations were also identified between the CK-18 and transaminase levels in the T2DM and NAFLD cohorts. Among the T2DM patients, the area under the ROC curve (AUROC) analysis indicated that the CK-18 level was the best serum predictor of NAFLD (0.75, 95%CI 0.67–0.81 compared with 0.73, 95%CI 0.65–0.79 for ALT, and 0.62, 95%CI 0.54–0.70 for AST). The best cutoff point predicting NAFLD was 180.93U/L, with a sensitivity of 44% and a specificity of 97%. Δ CK-18 was found to be significantly associated with Δ BMI in the T2DM patients with NAFLD.

Conclusion: The CK-18 level and the severity of NAFLD was positively correlated in the T2DM patients; thus, the CK-18 level is a potentially useful biomarker for assessing not only the severity but also the efficacy of treatment and the improvement in NAFLD in patients with T2DM.

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Unexpected prevalence of NAFLD among metabolically healthy patients with massive obesity

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Background and aims: Metabolically healthy obese (MHO) is a phenotype referring to obese patients who are relatively insulin sensitive, normotensive and have favourable glucose and lipid profiles. However, clinical and biological parameters defining this group remain imprecise. In addition, data concerning the hepatic status of these patients and the presence or absence of non alcoholic liver disease (NAFLD) are scarce. It is widely agreed that NAFLD contributes to insulin resistance and poor metabolic prognosis. To

evaluate the prevalence and the extent of NAFLD in MHO patients eligible for bariatric surgery

Materials and methods: In a prospective cohort of morbid obese patients undergoing bariatric surgery. Surgical liver biopsies were obtained from the left hepatic lobe at the beginning of different laparoscopic procedures, allowing sufficient histological analysis. The study was approved by the ethical committee, and all included patients signed informed consent. Currently, 66 patients were included (54 women), aged 36.4 ± 9.6 years, body mass index 44.8 ± 5.3 kg/m². All patients were free of other liver disease (alcohol, viral...). Liver changes were classified by histological analysis into 2 Groups (according to Bedossa and al 2012): normal liver, and NAFLD including simple steatosis and steatohepatitis (NASH). All clinical and biological data were collected: comorbidities, liver, lipid and glycemic profile, and HOMA-IR and fatty liver index (FLI) were calculated. MHO phenotype was defined as no or only one feature of the metabolic syndrome (IDF criteria)

Results: Of the 66 patients 22 had definite MHO status. Ten of them had histologically normal liver and 12 had NAFLD (9 steatosis and 3 NASH). In the metabolically unhealthy obese patients (44 MUO), 6 had histologically normal liver and 38 had NAFLD (25 steatosis, 13 NASH). Among MHO patients, those with NAFLD had higher BMI than patients with histologically normal liver (47.4 ± 6.4 vs 41.6 ± 3.8 kg/m², $p = 0.03$) while ALAT, ASAT, GGT, fasting plasma glucose and insulin, FLI index, HOMA-IR were mostly normal and similar

Conclusion: The prevalence of liver lesions is high in massive obesity, even in metabolically healthy patients, with no simple predictive marker except a higher BMI

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Combination of empagliflozin and linagliptin shows promise in a rodent model of non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are gaining increasing importance as the hepatic manifestations of metabolic syndrome and as primary causes for developing severe liver disease. Dipeptidyl peptidase (DPP)-4 and sodium-dependent glucose cotransporter (SGLT)-2 inhibitors are established treatments for type 2 diabetes (T2D). The DPP-4 inhibitor linagliptin (LINA) has been shown to improve hepatic steatosis and insulin sensitivity and reduce inflammation in preclinical models. The SGLT-2 inhibitor empagliflozin (EMPA) has been shown to improve insulin sensitivity of the liver, and improve several inflammatory markers in tissue of diabetic animal models. Here we investigated the effects of the combination of EMPA and LINA (EMPA+LINA) on hepatic parameters in db/db mice.

Materials and methods: We determined the effects of 8 weeks' treatment with EMPA (10 mg/kg/day) and LINA (3 mg/kg/day), alone or in combination, and vehicle on whole body insulin sensitivity in female db/db mice ($n = 15$ /group) using euglycaemic-hyperinsulinaemic clamps. Due to the mechanism of action of SGLT-2 inhibitors, euglycaemic-hyperinsulinaemic clamps were performed 4 days after the end of drug treatment. Liver triglyceride content was determined and inflammatory markers (F4/80 and suppressor of cytokine signaling [SOCS]-3) were detected using RT-PCR.

Results: Insulin-mediated suppression of hepatic glucose production (HGP) was significantly greater in the EMPA (13.1 mg/kg/min; $p < 0.05$) and EMPA+LINA groups (11.8 mg/kg/min; $p < 0.05$) compared with vehicle (26.3 mg/kg/min). LINA monotherapy decreased HGP (21.8 mg/kg/min), although statistical significance was not achieved. Tissue-specific labelled glucose uptake in liver was higher with EMPA (822 dpm/g/ml; $p < 0.05$), LINA (935 dpm/g/ml; $p < 0.05$), and EMPA+LINA (1040 dpm/g/ml; $p < 0.01$) compared with vehicle (610 dpm/g/ml). The glucose disposal rate was improved in the EMPA (5.9 mg/kg/min; $p < 0.001$), LINA (3.4 mg/kg/min; $p < 0.01$), and EMPA+LINA groups (7.8 mg/kg/min; $p < 0.001$) compared with vehicle (1.9 mg/kg/min). Glucose uptake into muscle and adipose tissue was not affected by any treatment. Compared with vehicle (14.6%), triglyceride content was significantly lower with EMPA (9.1%; $p < 0.05$), LINA (12.1%; $p < 0.01$), and EMPA+LINA (5.7%; $p < 0.01$) compared with vehicle. Levels of F4/80 and SOCS-3 mRNAs in the liver were reduced in the combination treatment compared with control.

Conclusion: The combination of EMPA+LINA was superior to the respective monotherapies in improving insulin sensitivity of the liver and in reducing liver lipid content. These results suggest that the combination of both drugs may be a potential therapy for the most common liver diseases associated with T2D.

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Mosapride citrate improves non-alcoholic steatohepatitis with increased faecal lactic acid bacteria and plasma glucagon-like peptide-1 level in a rodent model

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Background and aims: Several lines of evidence have suggested a role of gut microbiota in the etiology of non-alcoholic steatohepatitis (NASH). Gastrointestinal motility may influence gut microbiota and NASH subjects reportedly had a prolonged orocecal transit time coexistent with small intestinal bacterial overgrowth. Thus, we investigated effects of the gastroprokinetic agent mosapride citrate (MC) on gut microbiota and the development of NASH using methionine-choline deficient diet (MCDD)-induced NASH rodent model.

Materials and methods: 6-week-old C57BL/6 mice were divided into three groups, given the normal chow diet (NCD), the MCDD or the MCDD containing 10 mg/kg/day MC (MCDD plus MC) for 6 weeks.

Results: Gut microbiota analyses revealed that total numbers of bacteria were lower in the MCDD than in the NCD group, but similar to those in the MCDD plus MC group. Calculation of the relative abundance of each strain indicated lactic acid bacteria such as *Bifidobacterium* and *Lactobacillus* in feces to be specifically decreased in the MCDD group. Interestingly, the reduction in lactic acid bacteria in the MCDD group was reversed in the MCDD plus MC group. Subsequently, HE staining of livers from the MCDD group revealed remarkable NASH development as evidenced by deformity of hepatocytes, large fat droplets and inflammatory cell infiltration, all of which were suppressed in the MCDD plus MC group. Serum ALT levels were also elevated in the MCDD group, but were normal in the MCDD plus MC group. Azan staining revealed marked collagen deposition and mRNA levels of fibrosis markers such as α -smooth muscle actin, tissue inhibitor of metalloproteinase 1 and transforming growth factor β were elevated in the MCDD group, while these abnormalities were significantly reversed in the MCDD plus MC group. Finally, the molecular mechanism underlying the resistance to NASH development conferred by MC treatment was investigated. The mRNA level of tumor necrosis factor α and the serum concentration of lipopolysaccharide, a possible inducer of hepatic inflammation, were increased in the MCDD group, while these increases were suppressed in the MCDD plus MC group. Glucagon-like peptide-1 (GLP-1) reportedly attenuated the development of NASH. Plasma GLP-1 levels were lower in the MCDD than in the NCD group. Interestingly, plasma GLP-1 levels were increased in the MCDD plus MC group. To evaluate intestinal inflammation, immunostaining employing the anti-NF κ Bp65 antibody was performed. Nuclear NF κ Bp65 positive cells in the colon were increased in the MCDD group, while the numbers of these cells were reduced in the MCDD plus MC group.

Conclusion: MC showed a protective effect against the NASH development induced by MCDD, in which increased fecal lactic acid bacteria and plasma GLP-1 may be involved. Thus, MC may be effective for treating NASH.

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PKD2 deficiency reduces hepatic steatosis and insulin resistance in mice fed a high fat diet

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Background and aims: Hepatic steatosis is rapidly evolving as a major medical problem in the world. It involves a variety of lipid abnormalities including enhanced fatty acid influx from the adipose tissue, increased *de novo* lipogenesis, reduced fatty acid oxidation and ketogenesis. The pyruvate dehydrogenase kinases (PDKs) regulate pyruvate oxidation by controlling the activity of the pyruvate dehydrogenase complex (PDC). We examined whether PDKs were increased in the liver of HFD-fed mice and how they regulate hepatic steatosis and insulin resistance.

Materials and methods: The physiological importance of regulation of PDC activity by PDK isoenzyme 2 was assessed by comparing PDK2 knockout (PDK2 KO) mice with wild type mice fed a high fat diet (HFD) and an isocaloric low fat diet (LFD).

Results: Body weight gain and hepatic steatosis were attenuated by PDK2 deficiency in the HFD fed mice. Fasting blood glucose, serum insulin, and liver pyruvate, lactate, oxaloacetate, citrate, diacylglycerols, and triacylglycerols were also reduced. Hepatic glucose production was also reduced and insulin sensitivity was increased in the HFD fed PDK2 KO mice. The hepatic enzyme capacity for fatty acid oxidation and ketogenesis was increased while the capacity for lipogenesis was decreased. In spite of this but consistent with greater PDC activity, the respiratory exchange ratio was higher in the PDK2 knockout mice. Energy expenditure was increased without changes in physical activity. Increased hepatic insulin sensitivity and improved glucose tolerance correlated with reduced PKC ϵ phosphorylation.

Conclusion: The findings support the case for PDK2 as a promising target for hepatic steatosis and insulin resistance.

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Pro- and antioxidant status in dependence on vitamin D₃ availability in the development of diabetes-induced liver injury

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Background and aims: Diabetes is known to be associated with overproduction of ROS and/or impaired antioxidant defence contributing to the onset and progression of diabetic complications on vessels, retina, kidneys, nerves and liver. Nevertheless, precise mechanism by which oxidative stress could facilitate and accelerate the development of hepatic lesions in diabetes is not fully clarified. Recent studies have shown that vitamin D₃ is currently recognized as a potent immunomodulator and antioxidant affecting various inflammatory and autoimmune diseases. The present study was performed to determine the relationship between 25-hydroxyvitamin D₃ (25(OH)D₃) availability and pro-/antioxidant profile in liver of diabetic mice.

Materials and methods: Type 1 diabetes was induced in male C57BL/6 mice (weighing 25.0 \pm 1.5g) by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and STZ-diabetic mice were treated with or without vitamin D₃ (15 IU/mouse per os, for 8 weeks). Serum 25OHD₃ was assessed by ELISA. The levels of phospho-NF- κ B/p65, poly(ADP-ribose)/polymerase 1 (PARP-1), poly-ADP-ribosylated and nitrosylated proteins were measured by Western-blot analysis. Intracellular reactive oxygen and nitrogen species (ROS and RNS) production were detected by 2',7'-dichlorofluorescein (DCF) and 4,5-diamino-fluorescein diacetate (DAF-DA) fluorescence respectively using flow cytometry. Hepatic pro-/antioxidant factors and enzymes activity in liver were measured spectrophotometrically.

Results: Serum level of 25OHD₃, the main circulating metabolite of D₃, was shown to be reduced to 23.8 \pm 1.9 in diabetes vs. 39.7 \pm 2.9 nmol/l in control, that reflects reliably vitamin D₃ deficiency (p<0.05). As a strong evidence of diabetes-induced oxidative stress that may lead to liver lesions, increased hepatocytes ability to oxidize the fluorogenic substrate DCF and DAF was found. These changes were accompanied by a significant rise in the levels of protein nitrotyrosine, carbonyl groups and PAR by 42, 38, and 61% respec-

tively vs. control, $p < 0.05$. Diabetes also caused more than 1.67-fold increase in the level of 89 kDa apoptotic cleavage fragment of PARP. It was established diabetes-associated increase in the activities of key pro- and antioxidant enzymes in the liver: catalase (20%), SOD (7%), GPX (41%), xanthine oxidase (27%) DT-diaforase (54%) and NADPH-oxidase (39%). Alterations in pro-/antioxidant status in diabetes were paralleled by 1.51- and 2.04-fold over-expression of cytosolic and nuclear NF- κ B/p65 respectively as compared to control ($p < 0.05$), indicative of NF- κ B-mediated signaling pathways contribution to hepatic oxidative stress and inflammation. Vitamin D3 treatment completely restored blood serum 25OHD3 level, partially decreased PARP-1 and NF- κ B/p65 expression and counteracted diabetes-induced abnormalities of pro-/antioxidant profile in liver tissue. Normalized 25OHD3 availability strongly correlated with a significant decrease in ROS and RNS generation in hepatocytes as compared with diabetic mice.

Conclusion: The findings indicate that diabetes-associated vitamin D3 insufficiency can be related, at least in part, to increased prooxidant status of liver cells. Our data suggest a potential role of vitamin D3 treatment in the regulation of impaired oxidative metabolism in diabetes.

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Liver-specific deletion of mitochondrial prohibitin-2 alters glucose homeostasis associated with liver damage

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Background and aims: Regulation of mitochondrial dynamics and functions depends on various proteins. Recent studies highlighted the role of prohibitins which are highly conserved and ubiquitously expressed as two interdependent isoforms (Phb1 and Phb2). Prohibitins are found in the inner mitochondrial membrane of mitochondria and form large ring complexes. Their dysfunctions are associated with aging, cancer, obesity and inflammation. Mice lacking liver Phb1 exhibit hepatic injury and hepatocellular carcinoma. Recently, it showed that Phb2 is essential to maintain beta-cell mitochondrial function and integrity, thereby controlling insulin secretion and beta-cell survival. The present study focused on the role of Phb2 in liver biology using transgenic mice with liver-specific and time-controlled deletion of Phb2 (Hep-Phb2^{-/-}).

Materials and methods: Conditional liver-specific (Alb-Cre-ERT/lox) deletion of Phb2 was induced in 9-week old mice by sub-cutaneous implantation of one tamoxifen pellet per animal. After 3 weeks, mice were sacrificed, body and liver weights were measured and liver tissues analyzed by histology. The levels of hepatic transaminases (ALT and AST), bilirubin, cholesterol, triglycerides and glucose were determined in blood and glycogen content was measured in liver. Pyruvate tolerance test was also performed. Expression of Phb2, Phb1 and proteins involved in glycogenesis/glycogenolysis/gluconeogenesis were assessed by Western Blot.

Results: Three weeks after Tamoxifen implantation, hepatic levels of Phb2 protein were reduced by 90%. This deletion was associated with a 70–80% decrease of hepatic Phb1 protein. The body and liver weights of Hep-Phb2^{-/-} were significantly lower compared to control (Phb2fl/fl) mice. Levels of AST (642±94 vs 90±19 IU/L, $p < 0.001$), ALT (247±41 vs 33±3 IU/L, $p < 0.001$) and bilirubin (45±13 vs 2±0.1 μ M, $p < 0.05$) were increased in Hep-Phb2^{-/-} animals versus controls. Plasmatic levels of triglycerides (0.07±0.1 vs 1.1±0.1 mM, $p < 0.05$) and cholesterol (1.40±0.2 vs 1.9±0.07 mM, $p < 0.01$) were lower in Hep-Phb2^{-/-} mice compared to Phb2fl/fl mice. H&E staining showed pronounced histologic damages in Hep-Phb2^{-/-} such as bile duct hyperplasia, fibrosis and accumulation of lipid droplets. In fed conditions, blood glucose levels were lower (4.5±0.6 vs 7.8±0.8 mM, $p < 0.005$) in Hep-Phb2^{-/-} mice compared to Phb2fl/fl mice. Pyruvate tolerance test revealed impaired hepatic glucose production in Hep-Phb2^{-/-} mice. The hepatic glycogen content was reduced by 80% in Hep-Phb2^{-/-} animals versus Phb2fl/fl mice. Accordingly, marked reduction of expression of glucose-6-phosphatase (-25%), glucokinase (-90%), glycogen phosphorylase (-75%) and glycogen synthetase (-50%) were observed in liver of Hep-Phb2^{-/-} mice.

Conclusion: Hepatic deletion of Phb2 induced liver damage associated with impaired hepatic glycogen metabolism and hypoglycaemia. Liver Phb2 is required for the fine tuning of glycogenesis, glycogenolysis and gluconeogenesis, ultimately participating to glucose homeostasis.

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Increased inflammatory and procoagulant state are responsible for vascular complications in diabetic patients with albuminuria accompanied by fatty liver

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Background and aims: Vascular complications in diabetes are characterised by inflammation and a procoagulant state due to hyperglycaemia. The aim of this study was to compare the behaviour of inflammatory and other markers responsible for endothelial dysfunction and atherosclerosis related to nephropathy and liver steatosis in type 2 diabetic patients.

Materials and methods: Markers of inflammation and metabolic syndrome, were tested in 594 patients according to the presence of non-alcoholic fatty liver disease (NAFLD) and the albumin excretion rate (AER) (normoalbuminuria <30 mg/24h; albuminuria: 30–300 mg/24h). NAFLD was diagnosed by ultrasonography. Fatty liver index (FLI) (≤ 60 ; > 60) was used as a predictor of liver steatosis ($FLI = [e^{(0.953 \times \log_e(\text{triglycerides [Tg]}) + 0.139 \times \text{body mass index [BMI]} + 0.718 \times \log_e(\text{gamma-glutamyl transpeptidase [GGT]}) + 0.053 \times \text{waist-to-hip ratio [WHR]} - 15.745)} / 1 + e^{(0.953 \times \log_e(\text{Tg}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{WC} - 15.745)}] \times 100$). AER was measured from three urine samples. Peripheral insulin sensitivity was measured using estimated glucose disposal rate ($eGDR = 24.31 - [12.22 \times \text{WHR}] - [3.29 \times \text{hypertension}] - [0.57 \times \text{glycated haemoglobin (HbA1c)}]$). Patients were divided into quartiles according to FLI and AER (1st: FLI ≤ 60 and AER <30, 2nd: FLI >60 and AER <30, 3rd: FLI ≤ 60 and AER:30–300 and 4th: FLI >60 and AER:30–300).

Results: Significant differences ($p < 0.05$) were determined using analysis of variance in waist circumference (WC), postprandial blood glucose (BGPP), HbA1c, adiponectin (ApN), fibrinogen (FIB), white blood cell (WBC) count, AER, high density lipoprotein (HDL), Tg, alanine transaminase (ALT), fasting C-peptide (FC) and eGDR among the groups according to the presence of NAFLD and albuminuria. Tested groups that differed were determined using Tukey post hoc test. Patients in the 4th quartile had significantly higher FIB, WBC, WC, BGPP, HbA1c, Tg, ALT, and FC-peptide, and lower ApN, HDL and eGDR values compared with the 1st quartile. Patients in the 2nd quartile had higher WC and lower eGDR compared with the 1st quartile. After stepwise regression procedure for FIB as a dependent variable, the best model included ($R^2 = 0.479$) high-sensitivity C-reactive protein (hs-CRP), systolic blood pressure (SBP), WHR, WBC and ApN. The best model for WBC ($R^2 = 0.310$) included FLI and interleukin-6 (IL-6). FIB and WBC correlated significantly ($p < 0.05$) with hs-CRP, IL-6, BGPP, HbA1c, AER, HDL, uric acid, FLI and eGDR; FIB additionally correlated with fasting BG, and WBC with WC, ApN and Tg.

Conclusion: Increased FIB, WBC, Tg, ALT, BGPP and HbA1c, and decreased ApN, HDL and eGDR pointed to increased inflammatory and procoagulant state in the presence of albuminuria and NAFLD. Decreased eGDR reflected increased insulin resistance even in normoalbuminuric patients with NAFLD. Reported correlations among the tested variables unravelled the association between inflammation, procoagulant state and glycaemia as a culprit of vascular complications in the studied patients.

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Hepatic heat shock proteins in long term diabetic complications

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Background and aims: Current regimes for the treatment of Diabetes mellitus type 2 (DM2) can only postpone, but not prevent the development of long term diabetic complications. Therefore, the continuously earlier age of DM2 onset will dramatically increase the incidence and severity of diabetic complications in future years. The course of DM2 complications includes accumulation of damaged, misfolded and aggregated proteins, while protective heat shock proteins (HSP) are repressed. With this study we aim to identify HSP-regulated liver-to-periphery signalling pathways effecting the development and progression of long term diabetic complications.

Materials and methods: Livers of Streptozotocin-induced type 1 diabetic mice, obesity-induced type 2 diabetic mice (db/db), mice fed a high-fat diet

and respective control mice were analyzed for expression of HSPs by qPCR and western blot. Hepatocyte-specific reconstitution of HSPs in db/db mice was achieved via adenoviral constructs for Hsp70, DNAJB1 and DNAJA2.

Results: In our models of diabetes, we observed downregulations of up to 90% in the hepatic expression of central HSPs such as Hsp70. In contrast, non-diabetic mice on a high-fat diet showed no significant regulation of hepatic HSP expression. In db/db mice, two weeks of hepatocyte-specific reconstitution of Hsp70 reduced both fasting glucose and HbA1c by approx. 25% ($p<0.005$) along with improved liver histopathology. Parameters of lipid metabolism were not significantly affected. Since efficient recognition and processing of damaged and aggregated proteins requires the cooperation of HSPs from different HSP families, we also analyzed the *in vivo* effects of simultaneous reconstitution of the cooperating HSPs Hsp70, DNAJB1 and DNAJA2 in db/db mice. This combined reconstitution improved fasting blood glucose from 444 ± 91 mg/dl in control mice to 269 ± 101 mg/dl in HSP-treated mice ($\sim 40\%$, $p<0.005$). As a measure of overall liver pathology, serum alanine amino transferase (ALT) dropped from 325 ± 112 IU/l to 108 ± 50 IU/l ($p<0.005$). The response to acute glucose challenge showed a highly significant reduction of glucose tolerance ($p<0.001$ in two way ANOVA). Along with reduced serum insulin levels in HSP-treated mice, the improved glucose tolerance test was suggestive for a reduced insulin resistance and increased glucose uptake in periphery organs like muscle and adipose tissue. Importantly, only six days after reconstitution of cooperating HSPs, the thermal nociceptive responsiveness was significantly improved from 32.5 ± 11 sec. in control mice to 24.5 ± 8 sec. in HSP-treated mice ($p<0.01$). Peripheral nerve histology revealed a significant change in the number of infiltrating leukocytes, pointing towards immunological mechanisms that reduced the pre-existing diabetic neuropathy.

Conclusion: The hepatic reconstitution of HSPs cooperating in protein quality control resulted in a profound metabolic improvement in our *in vivo* model of DM2, affecting not only parameters of carbohydrate metabolism but also insulin resistance and the long term complication of diabetic neuropathy. Our data indicate that hepatic HSPs regulate systemic signalling pathways which direct the course of diabetes and its long term complications.

Supported by: Dietmar Hopp Stiftung

and extra-large HDL correlated positively with hepatic glucose uptake rate ($r=0.542$; $p=0.025$ and $r=0.510$ and $p=0.036$, respectively) and these particles associated negatively with hepatic glucose production ($r=-0.624$, $p=0.013$ and $r=-0.547$ and $p=0.035$, respectively). Results from the liver genetic data are in process.

Conclusion: We showed that surgery induced, rapid weight loss caused significant increases in antiatherogenic large HDL subclasses and that these were associated with hepatic glucose metabolism. In addition, this study used human liver samples, which are restricted based on ethical rationales. This pioneer study builds a framework for understanding hepatic lipoprotein metabolism in morbid obesity, a condition preluding insulin resistance and type 2 diabetes. With our novel findings, early prevention strategies can be induced. In summary, this report delivers an in-depth conceptualization about how liver and lipoprotein synergy dictates diabetes-induced cardiovascular risk factors.

Supported by: AOF

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Lipoprotein subclasses associate with hepatic glucose uptake, production, and genetic targets after bariatric surgery in morbidly obese patients

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Background and aims: The cardiovascular complications arising from morbidly obese individuals remains unsettled. If lifestyle modifications are not effective in reducing weight, these individuals have a high risk for insulin resistance and diabetes. Bariatric surgery is a potent treatment to rapidly remove weight. Although absolute very-low-density lipoprotein cholesterol (VLDL) serum levels are decreased and high-density lipoprotein (HDL) cholesterol concentrations are increased after bariatric surgery, their heterogeneity/subclass distributions remain unclear. These subclasses play a major role in the extent of atherosclerosis severity. This study's objective was to map these subclasses in morbidly obese participants, both pre- and post-surgery, and to assess hepatic metabolic and genetic associations.

Materials and methods: Hepatic glucose uptake and production were ascertained using ¹⁸F-fluorodeoxyglucose and positron emission tomography from 23 morbidly obese (BMI > 39 kg/m²) participants, before and six months after surgery. Ten healthy lean controls were also recruited. Liver biopsies were taken from 23 participants before surgery. Targeted, real-time PCR analysis was conducted to assess gene levels in the morbidly obese, on lipoprotein assembly and catabolic genes: microsomal triglyceride transfer protein, apoB, apoE, ABCA1, apoA1, LDL receptor, CD36, hepatic lipase, and LCAT. Genes involved with liver triglyceride metabolism such as glucose kinase regulatory protein, DGAT, etc. were also profiled. Lipoprotein subclass profiles, from serum, were analyzed by NMR spectroscopy. Principal component analysis explained the 95 percent variation in the dataset. After a Bonferroni correction, p-values less than 0.001041 were significant. Lipoprotein subclasses, which changed significantly, were compared to hepatic glucose uptake, production, and genetic profiles using Pearson correlations.

Results: After bariatric surgery, large, medium, and small VLDL-cholesterol concentrations were significantly reduced, while extra-large and large HDL cholesterol subclasses were significantly higher. Only after surgery, large

PS 116 Vascular calcification

1284

Oestrogen-related receptor gamma mediates vascular calcification through up-regulation of BMP2

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Background and aims: Vascular calcification, which refers to ectopic mineralization in vascular smooth muscle cells, occurs frequently in many diseases such as chronic kidney disease, atherosclerosis, and diabetes. Estrogen related receptor (ERR) γ , a member of orphan nuclear receptor superfamily has diverse roles in regulating homeostatic and metabolic processes. However the role of ERR γ in vascular calcification has not yet been investigated. This study was undertaken to examine the role of ERR γ in vascular calcification.

Materials and methods: Rat aortic smooth muscle cells (RASMCs) were cultured and vascular cell calcification was induced by treatment with inorganic phosphate and calcium. Next we investigated effects of adenovirus-mediated overexpression of ERR γ on phosphate-induced VSMC calcification and examined whether siRNA-mediated inhibition of endogenous expression of ERR γ or pharmacological inhibition of ERR γ with GSK5182 prevent phosphate-induced vascular calcification.

Results: Along with increased expression of bone morphogenic protein-2 (BMP2), Runx2 and Msx2, ERR γ expression was upregulated during phosphate - induced calcification. Von-kossa staining showed that adenovirus-mediated overexpression of ERR γ (Ad-ERR γ) in RASMCs accelerated Pi-induced calcification. Ad-ERR γ increased the expression of osteogenic gene including Runx2, OPN and Msx2 but decreased α -SMA. ERR γ induced BMP2 transcription through directly binding to its promoter region and increased BMP2 signaling including phosphorylation and nuclear localization of smad1/5/8. Moreover, inhibition of ERR γ by both siRNA-mediated knockdown of endogenous ERR γ and a selective inverse agonist, GSK5182 attenuated vascular calcification and osteogenic gene expression in vitro and in vivo.

Conclusion: This study demonstrated that ERR γ mediates vascular calcification through up-regulation of BMP2 signaling. These results indicate that inhibition of ERR γ may be a potential therapeutic strategy for prevention of vascular calcification.

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Effects of high glucose on the OPG/RANK/RANKL/TRAIL system in the progression of vascular calcification in rat aortic vascular smooth muscle cells

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Background and aims: Diabetes mellitus is frequently complicated by cardiovascular disease, such as vascular calcification and accelerated atherosclerosis. Recently, it has been known that the OPG/RANK/RANKL/TRAIL system may play a major role in vascular calcification and atherosclerosis. However, the possible effects of long term high glucose stimulation at least 4 weeks on the OPG/RANK/RANKL/TRAIL system in the progression of vascular calcification are less clear. We attempted to evaluate the effect of high glucose on the progression of vascular calcification in rat aortic smooth muscle cells (RASMCs) and to detect the expression changes of OPG, RANK, RANKL, and TRAIL for 2 and 4 weeks. Furthermore, we used BMP-7, which has been known to attenuate vascular calcification, to detect the possible changes of OPG, RANK, RANKL, and TRAIL expressions on the calcification of RASMCs.

Materials and methods: The primary cultured RASMCs were stimulated with normal glucose (5.5mmol/L glucose, NG) and high glucose (30 mmol/L glucose, HG) with calcification medium. The mRNA levels and the protein expressions of OPG, RANK, RANKL, and TRAIL were measured by reverse transcription polymerase chain reaction (RT-PCR) or Western blot.

Results: The intensity of calcium staining was increased in HG after 2 weeks and more increased and prominent after 4 weeks compared to NG. OPG mRNA and protein expressions were not different after 2 weeks, however, after 4 weeks, OPG expressions were significantly decreased in HG. Regarding RANK, RANKL, and TRAIL expressions, there were no differences after 2 or 4 weeks of stimulation. After 4 week of rhBMP-7 co-treatment, the densities of calcium stains were attenuated and the total amount of calcium was also decreased. The mRNA and protein OPG expressions were maintained with BMP-7 after 4 weeks of stimulation. There were no differences in the expressions of RANK, RANKL, and TRAIL between with and without BMP-7 co-treatment. There was no difference in Bax mRNA expression, an apoptotic marker, between with and without BMP-7 co-treatment, however, ALP mRNA expression, a marker of mineralization, was decreased in the presence of BMP-7.

Conclusion: Chronic hyperglycemia may enhance vascular calcium deposition and high glucose may increase OPG mRNA and protein expression with short-term stimulation, but decrease it with long-term stimulation. The expression of RANKL, RANK, and TRAIL were maintained with long term stimulation. As the OPG expression was decreased, the increased mineralization activity may be more associated rather than apoptotic activity with the progression of long-term high glucose induced vascular calcification.

1286

The DPP-4 inhibitor linagliptin increases plasma fetuin-A concentrations in a rat model of uraemic calcification

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Background and aims: Fetuin-A is a potent inhibitor of ectopic mineralisation. An increase in fetuin-A has beneficial effects in uraemic vascular calcification, whereas fetuin-A deficiency is associated with soft tissue calcification in mice and humans. Biomarker data in diabetic and non-diabetic rodent models of chronic renal failure suggest that the dipeptidyl peptidase (DPP)-4 inhibitor linagliptin may be able to influence the pathogenesis of vascular calcification. The aim of the present study was to investigate the effect of linagliptin on uraemic vascular calcification in nephrectomised rats.

Materials and methods: 1,25-dihydroxyvitamin D3 (0.25 μ g/kg/day) was used to induce uraemic vascular calcification in 5/6 nephrectomised rats (5/6NxVitD). Rats were allocated to 3 treatment groups: a) sham-operated rats treated with placebo (n=10); b) 5/6NxVitD rats treated with placebo (n=14); c) 5/6NxVitD rats treated with linagliptin (n=14). Rats were treated for 6 weeks. We analysed plasma factors known to be involved in uraemic vascular calcification such as cystatin C, fibroblast growth factor 23 (FGF23), magnesium, calcium, phosphate, and fetuin-A. Blood pressure was monitored and at study end, animals were sacrificed; blood was taken and stored at -80°C for analysis.

Results: Blood pressure was similar in all groups. 5 of 14 animals died in the 5/6NxVitD placebo group; 3 of 14 animals died in the 5/6NxVitD linagliptin group ($p>0.05$). At study end, magnesium, FGF23, and cystatin C levels were unaffected by linagliptin in 5/6NxVitD rats. The change from baseline in phosphate levels was similar with linagliptin and placebo in 5/6NxVitD rats, whereas the change from baseline in calcium levels was less with linagliptin (-0.062 ± 0.255 mmol) than placebo (0.961 ± 0.439 mmol) in 5/6NxVitD rats ($p<0.05$). The change from baseline in fetuin-A levels was greater with linagliptin (1.278 ± 0.125 g/l) than placebo (0.919 ± 0.072 g/l) in 5/6NxVitD rats ($p<0.05$).

Conclusion: The favorable change from baseline in plasma calcium after 6 weeks of linagliptin treatment in an aggressive rat model of uraemic vascular calcification may be due to an increase in fetuin-A levels. Although further studies are required, linagliptin treatment may potentially offer a new therapeutic option to reduce uraemic vascular calcification in patients with advanced stages of renal failure.

Supported by: Boehringer Ingelheim

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Below-knee arterial calcification in type 2 diabetes: association with receptor activator of nuclear factor kappa B ligand, osteoprotegerin and neuropathyO. Bourron^{1,2}, C.E. Aubert¹, S. Liabeuf³, P. Cluzel¹, M. Komajda¹,S. Jacqueminet¹, Z. Massy³, A. Hartemann^{1,2};¹Pierre et Marie Curie University Paris 06 - Assistance Publique Hôpitaux De Paris, ²CHU Pitié-Salpêtrière,³Picardie University - CHU Amiens, Paris, France.

Background and aims: Calcification of the arterial wall in diabetes contributes to the arterial occlusive process at the below knee level. The osteoprotegerin(OPG)/RANKL system is suspected to be involved in the calcification process. The aim of the study was to investigate if there is a link between arterial calcification in type 2 diabetes and 1) the conventional cardio-vascular risk factors, 2) the serum RANKL and OPG levels and 3) neuropathy.

Materials and methods: We objectively scored, in a cross-sectional study, infra-popliteal vascular calcification using CT-scan in 198 patients with type 2 diabetes, a high cardio-vascular risk and with glomerular filtration rate > 30mL/mn. Colour duplex ultrasonography was performed to assess peripheral arterial occlusive disease, and mediocalcosis. Peripheral neuropathy was defined by a neuropathy disability score (NDS) > 6. RANKL and OPG were measured in serum by routine chemistry.

Results: Below knee arterial calcification was associated with arterial occlusive disease. In multivariate logistic regression analysis, variables significantly and independently associated with the calcification score were age (OR=1.08; 95% CI=1.04-1.13; p<0.0001), male gender (OR=3.53; 95% CI=1.54-8.08; p=0.003), previous CVD(OR=2.78; 95% CI=1.39-5.59; p=0.005) and NDS (per 1 point, OR=1.21; 95% CI=1.05-1.38; p=0.006). The association with Ln OPG, significantly associated with calcification score in univariate analysis (OR=3.14; 95% CI=1.05-9.40; p=0.045), was no longer significant in multivariate analysis. RANKL and OPG/RANKL were not significantly associated with the calcification score.

Conclusion: Below knee arterial calcification severity is clearly correlated with peripheral neuropathy severity and several usual cardio-vascular risk factors, but not with serum RANKL level.

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Serum fetuin levels are associated with peripheral arterial disease in patients with type 2 diabetes mellitusI. Eleftheriadou¹, P. Grigoropoulou¹, I. Mourouzis², C. Liaskos¹,A. Kokkinos¹, D. Perrea³, K. Makrilakis¹, N. Katsilambros¹, N. Tentolouris¹;¹First Department of Propaedeutic and Internal Medicine, Athens University Medical School, Laiko General Hospital, ²Department of Pharmacology, Athens University Medical School, ³Laboratory of Experimental Surgery and Surgical Research, Athens University Medical School, Greece.

Background and aims: Fetuin is a hepatic glycoprotein that inhibits arterial calcification and insulin action. Patients with type 2 diabetes mellitus (T2DM) have higher fetuin levels than non-diabetic individuals. However, increased fetuin levels have been associated with lower cardiovascular risk among non-diabetic individuals, while a trend towards the opposite direction has been demonstrated in patients with T2DM. Conflicting data exists regarding the association between fetuin and peripheral arterial disease (PAD) in diabetes. The purpose of this study was to determine the relationship of serum fetuin levels with PAD in patients with T2DM.

Materials and methods: A total of 71 patients (142 feet) with T2DM were recruited (mean age 67.7±8.9 years, mean diabetes duration 15.0±10.6 years, male/female 45/26). Serum fetuin levels were measured using ELISA. Diagnosis of PAD was based on the presence of either biphasic, monophasic or blunted waveforms at the pedal arteries, while ankle-brachial index (ABI) was also measured.

Results: Patients with PAD (n=35) had significantly lower serum fetuin levels in comparison with those without PAD (510.0±108.5 vs 562.3±121.2 µg/ml, p=0.008). Fetuin levels were significantly associated with ABI (r=0.236, p=0.006). Univariate logistic regression analysis showed that age (p=0.004), male gender (p=0.022), diabetes duration (p=0.001), dyslipidemia (p=0.002), arterial hypertension (p=0.008), smoking (p=0.052) and fetuin levels (p=0.010) were significantly associated with PAD. No significant associations were found with BMI and HbA1c. Multivariate logistic regression analysis,

after adjustment for age and gender demonstrated that PAD was significantly associated with diabetes duration [odds ratio (OR): 1.06, 95% confidence intervals (CI): 1.01-1.10, p=0.014], dyslipidemia (OR: 5.2, 95% CI: 1.85-14.57, p=0.002), arterial hypertension (OR: 7.0, 95% CI: 1.55-32.13, p=0.012), smoking (OR: 5.0, 95% CI: 1.27-19.55, p=0.022) and fetuin levels (OR: 0.99, 95% CI: 0.989-0.997, p=0.001).

Conclusion: Serum fetuin levels are lower in patients with PAD and are associated with the presence of PAD irrespective of traditional cardiovascular risk factors.

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Relation of epicardial adipose tissue volume, microalbuminuria, thyroid hormones axis to coronary artery calcium in type 2 diabetic patientsM.M.M. Aboromia¹, H.A. Elorabi², K.M. Makbol³, Y.M. Eid²,S.A. Moharam³, A.M. Hamam³;¹Internal Medicine, Diabetes Unit, Ain Shams University Hospitals,²Internal Medicine, Diabetes Unit, Ain Shams University Hospitals,³Cardiology, Military Medical Academy, Cairo, Egypt.

Background and aims: Cardiovascular disease (CVD) is the most significant cause of mortality in type 2 diabetes mellitus (T2DM) patients. Visceral adipose tissue (VAT) may be important in sustaining the proinflammatory background of cardiovascular disease. Epicardial adipose tissue (EAT), is a special VAT that surrounds the major branches of the coronary artery branches and myocardium. This close anatomical relationship between EAT and coronary arteries and myocardium further promotes local paracrine interactions between these tissues. Calcific deposits in coronary artery are in connection with coronary artery atheromatous plaque and is considered an indicator of atherosclerosis. Microalbuminuria has long been recognized as an important biomarker that predicts micro and macrovascular complications and mortality in patients with T2DM. Complex interplay between thyroid function and insulin resistance has been implicated in diabetic dyslipidemia. Furthermore, triiodothyronine (T3) was found to be a key signal in myocardial cells. Whether there is an association between EAT, albuminuria, thyroid hormones to coronary atherosclerosis and coronary artery calcium is a question to be further evaluated. Objective: to investigate the relation between EAT volume, thyroid hormones and microalbuminuria to coronary artery calcium in T2DM.

Materials and methods: Design: This study was conducted on 100 T2DM patients, they were further divided according to their calcium score into 4 groups. Gr 1: T2DM with coronary artery calcium (CAC 0-10), Gr 2: T2DM patients with CAC (11-100), Gr 3: T2DM patients with CAC (101-400), Gr 4: T2DM patients with CAC >400, all patients were submitted to history taking, clinical and anthropometric evaluation, laboratory investigations (FBG, 2 h PP, lipid profile, HbA1c%, serum insulin, albumin creatinine ratio, TSH, free T3, free T4), HOMA-IR, coronary multislice CT.

Results: There was a high statistical significant difference between the 4 groups as regards EAT volume, being the highest (>200 cm³) among Gr 4, there was a high statistical significant difference between the 4 groups as regards HbA1c%, (10.23±1.49), Urinary albumin creatinine ratio (UACR) (109.87±52.31) mg%, (P<0.001) being the highest among Gr 4, there was a significant difference between the 4 groups as regards TSH levels (3.35±0.63) being the highest among Gr 4 (P<0.05). Correlation between CAC and different studied parameters showed a high statistical significant (P<0.001) direct correlation between CAC and Body mass index (r=0.396), total cholesterol (r=0.267), non HDL (r=0.274), fasting blood glucose (FBG) (r=0.363), 2 hours Post prandial blood glucose (2H PPg) (r=0.354) HbA1c% (r=0.510), TSH (r=0.293), UACR (r=0.515), EAT (r=0.831). A statistical significant (P<0.05) direct correlation between CAC and age (r=0.200), Serum triglycerides (r=0.216) HOMA-IR (r=0.245). A statistical significant (P<0.05) indirect correlations were found between CAC and HOMA-B (r=-0.222) and free T3 (r=-0.229).

Conclusion: CAC score is strongly related to EAT volume, blood glucose control, insulin resistance, albuminuria and thyroid axis hormones.

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Metabolically unhealthy non-obese subjects are at higher risk of subclinical coronary atherosclerosis than metabolically healthy obese subjects in KoreaK.-U. Lee^{1,2}, J. Jang^{1,2}, C. Woo¹, M. Lee¹, C. Jung¹, W. Lee¹, E. Koh^{1,2}, J.-Y. Park¹, I.-K. Lee³, M. Choi⁴;¹Department of Internal Medicine, Asan Medical Center, Seoul,²Metabolism Research Unit, Asan Institute for Life Sciences, Seoul,³Department of Internal Medicine, Kyungpook National University School of Medicine, Daegu, ⁴Department of Internal Medicine,

College of Medicine, Chuncheon, Republic of Korea.

Background and aims: Metabolically healthy obesity (MHO) is an emerging phenotype with a cardiovascular disease (CVD) risk between healthy, normal weight and unhealthy, obese individuals. It is not yet established whether this phenotype has higher risk of CVD than nonobese subjects with metabolic abnormalities. In this study, we compared the degree of subclinical coronary atherosclerosis detected by coronary multidetector computed tomography (MDCT) in four groups defined by the state of metabolic health and obesity in an asymptomatic Korean population.

Materials and methods: We collected the data of 4,009 asymptomatic subjects (mean age, 53.2 yr) who participated in a routine health screening examination at a medical center in Korea. Significant coronary artery stenosis (CAS) defined as >50% stenosis, and coronary artery calcium scores (CACS) were assessed by MDCT. Participants were stratified by BMI (cut-off value, 25 mg/m²) and metabolically healthy state.

Results: MHO subjects (n = 589) had a significantly higher prevalence of subclinical coronary atherosclerotic burden (CAS and CACS > 0) compared with metabolically healthy non-obese (MHNO) subjects (n = 1367). However, MHO subjects had a significantly lower prevalence of subclinical coronary atherosclerotic burden (CACS > 0) compared with metabolically unhealthy nonobese (MUNO) subjects (n = 853) as well as metabolically unhealthy obese (MUO) subjects (n = 1200). The prevalence of subclinical coronary stenosis in MUNO subjects was comparable to MUO subjects.

Conclusion: Our data show that MHO might not be a benign disease in terms of coronary atherosclerotic burden. However, MHO subjects had a significantly lower prevalence of subclinical coronary atherosclerotic burden compared with MUO subjects. Thus, the presence of metabolic abnormalities may be more important than the presence of obesity in evaluating cardiovascular risk.

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Contributors to mortality in high risk diabetes patientsD.W. Bowden¹, F.-C. Hsu², B.I. Freedman³, J. Carr⁴, A.J. Cox⁵;¹Center for Human Genomics, ²Biostatistical Sciences, ³Internal Medicine, Wake Forest University School of Medicine, Winston-Salem,⁴Radiology, Vanderbilt University School of Medicine, Nashville, USA,⁵Griffith Health Institute, Southport, Australia.

Background and aims: Individuals with type 2 diabetes (T2D) exhibit substantial differences in mortality risk. Coronary artery calcium (CAC) is a powerful, well documented, predictor of mortality. In this study we examined a set of individuals at high risk for death based on CAC>1000 (40% mortality in 7.5 years) and evaluated a range of clinical measures, including modifiable cardiovascular disease (CVD) risk factors, for insights into risk for mortality.

Materials and methods: All-cause mortality was determined in 371 European American individuals with T2D and CAC>1000 from a larger ongoing population based cohort study of CVD in people with T2D. After 8.2 ± 3.0 (mean ± SD) years of follow-up differences in CVD risk factors, medication use, and other clinical measures were compared between living (n=218) and deceased (n=153) participants. Cox Proportional Hazards Regression models were used to quantify risk for mortality to appropriately account for time-to-event effects.

Results: Mean age at baseline was 65.5 years with mean duration of diabetes 12.6 years. The subjects had a mean BMI of 32.0 and were 70.1% male. Death was confirmed through the US Social Security death index. Deceased participants had a longer duration of T2D (p=0.02). Differences in cholesterol were nominal (HR 2.24 CI 1.09–4.60; p=0.03), but kidney function (HR 4.78 CI 2.38–9.62; p<4x10⁻³) and CRP (HR 2.14 CI 1.33–3.43; p=0.002) were more strongly associated with mortality. Strikingly, measures of blood pressure, use of anti-hypertensive and hypoglycemic medications, and prior prevalent CVD were not significantly associated with risk. Use of cholesterol-lowering

medication was strongly associated with survival (HR 1.68 CI 1.19–2.39; p=0.004). Additional measures of subclinical disease revealed that a vascular calcification score derived from three arterial beds (coronary, carotid and aortic) was associated with the greatest risk for mortality (HR 2.31; p=3.43x10⁻⁷). In multivariable models elevated HbA1c, lipids, CRP and calcified plaque and lower kidney function were associated with a 1.1–1.5-fold increased risk for mortality (3.5x10⁻⁶ < p < 0.03), after adjustment for confounding factors.

Conclusion: Even in this high-risk group, a multi-bed assessment of vascular calcification and known CVD risk factors provide useful information for risk assessment. Importantly, even in a high risk T2D sample, patients have differential risks of death over a substantial time period and the use of cholesterol-lowering medication appeared protective for mortality.

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A diagnostic dilemma: an investigation of non-invasive vascular assessment of the lower extremity in people with diabetesV.H. Chuter¹, P.E. Craike¹, N.A. Johnson², S.L. Casey¹;¹Health Sciences, University of Newcastle, Ourimbah,²Health Sciences, University of Sydney, Lidcombe, Australia.

Background and aims: Peripheral arterial disease (PAD) is estimated to affect 21% of people over the age of 65. Non-invasive vascular assessment of the lower extremity using the ankle brachial index (ABI) is recommended to screen for the disease in those at risk of PAD including older people and people with diabetes. It is well known that the ABI loses clinical utility in the presence of diabetes and in older age due to the presence of medial arterial calcification (MAC). The toe-brachial index (TBI) is recommended as an alternative to the ABI where elevated ABI values suggest incompressible arteries due to MAC. However there is some research to suggest the TBI may be more clinically effective in all people with diabetes due to the presence of co-existent PAD and MAC producing normal ABI values in the presence of significant arterial pathology. The aim of this study was to investigate the comparative diagnostic accuracy of the TBI and ABI for the presence of PAD in people with and without diabetes.

Materials and methods: Participants meeting current guidelines for lower extremity vascular screening including people with diabetes were recruited on a volunteer basis from two university podiatry teaching clinics. ABI and TBI measurements were performed on the right lower extremity of all participants. Participants subsequently underwent colour duplex Ultrasound from the abdominal aorta to the distal ankle on the right side. This was used as the reference standard to calculate sensitivity, specificity and diagnostic accuracy of both the TBI and ABI for the presence of PAD. ROC analysis was performed to determine the clinical efficacy for diagnosis of PAD of each test.

Results: One hundred and sixty nine people were recruited to this study including 89 people with Type 2 diabetes and 100 males, with a mean age 73.57 years (SD 7.31 years). The ABI has the highest specificity for PAD in both people with and without diabetes (specificity 93%, 95%CI 0.82 to 0.98 and 95%, 95%CI 0.84 to 0.98 respectively). The TBI had greater sensitivity than the ABI in people with and without diabetes (sensitivity with diabetes TBI: 85%, 95%CI 0.72 to 0.92, ABI: 42%, 95%CI 0.30 to 0.57, sensitivity without diabetes TBI: 89%, 95%CI 0.75 to 0.96, ABI: 61%, 95%CI 0.45 to 0.75). Overall, and, in the presence of diabetes, diagnostic accuracy was higher for the TBI (TBI: 78% and 88% respectively, ABI: 68% and 79% respectively). ROC analysis indicated the TBI had greater clinical efficacy for the diagnosis of PAD in people with diabetes and in the entire study population (ROC area:0.82 p=.0001, and 0.81 p=0.0001) than the ABI (ROC area:0.59, p=0.09, and 0.67, p=0.0001).

Conclusion: Our results demonstrated that the ABI had greater specificity for the presence of PAD than the TBI in people with and without diabetes. However, overall the TBI demonstrated greater diagnostic accuracy and may be more clinically effective than the ABI for diagnosing PAD in people with diabetes and those meeting current guidelines for PAD screening, particularly if PAD is suspected. Further research needs to evaluate relationships between severity and location of PAD and the relative clinical utility of the TBI and ABI in people with diabetes.

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PS 117 Vascular dysfunction

1293

Caveolin-1 alpha expression is decreased in the aorta of rats with glucose intolerance induced by a low dose of streptozotocin

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Background and aims: Caveolae are plasma membrane invaginations of endothelial cells, which represent a predominant location of endothelial nitric oxide synthase (eNOS), important in regulation of endothelial function. Caveolin-1 (cav-1) is the main component of caveolae, but the specific role of its isoforms α and β in regulation of eNOS activity in diabetes and glucose impaired tolerance is still not known.

Materials and methods: Impaired glucose tolerance was involved by streptozotocin administration (STZ, 25 mg/kg/day, i.p. injections after every 24h, n=10). Control (CON, n=10) received the vehicle. After 8 weeks, oral glucose tolerance test (OGTT) was performed. At the end of experiment (10 weeks after STZ) we measured of vascular reactivity to acetylcholine. The expressions of cav-1 α , cav-1 β , eNOS, hsp90, gp91phox and MnSOD were analyzed by Western blot in aorta samples. The expressions of both isoforms of cav-1 in aorta were also determined at mRNA level by qRT-PCR.

Results: We observed normal fasting glucose in both groups (CON 6.15 \pm 0.27 mmol/l vs. STZ 6.65 \pm 0.97 mmol/l), but STZ group had a significantly increased glycaemia 1h after oral administration of glucose in OGTT (CON 8.06 \pm 0.44 mmol/l vs. STZ 16.75 \pm 2.58 mmol/l, $P < 0.05$). A decreased endothelium-dependent relaxation to acetylcholine was observed in STZ animals (pD₂: CON 6.71 \pm 0.16 vs. STZ 6.27 \pm 0.08, $P < 0.01$; Emax: CON 37.07% \pm 3.08 vs. STZ 29.67% \pm 1.42, $P < 0.01$), whereas endothelial-independent relaxation to nitroprusside was not changed. We found a decreased expression of eNOS in STZ group (CON 100 \pm 12.85 vs. STZ 39.48 \pm 7.14, $P < 0.05$), while the expressions of hsp90 (CON 100 \pm 17.36 vs. STZ 116.26 \pm 11.48), gp-91phox (CON 100 \pm 17.09 vs. STZ 139.43 \pm 21.53) and MnSOD (CON 100 \pm 11.21 vs. STZ 108.08 \pm 14.34) were not altered. There were no changes in the expression of both isoforms of cav-1 at mRNA level (cav-1 α : CON 1.00 \pm 0.12 vs. STZ 1.26 \pm 0.2; cav-1 β : CON 1.00 \pm 0.21 vs. STZ 1.39 \pm 0.18). However, we observed a significant downregulation of cav-1 α (CON 100 \pm 12.42 vs. STZ 32.22 \pm 12.06, $P < 0.01$) and a trend of increased expression cav-1 β at protein level in STZ group (CON 100 \pm 23.86 vs. STZ 149.49 \pm 13.86, $P = 0.07$).

Conclusion: Our result showed an impaired glucose tolerance after low-dose streptozotocin, which was associated with dysfunction of endothelial-dependent relaxation and decreased expression of eNOS. Endothelial dysfunction in STZ group probably is not related to expression of gp91phox and MnSOD. It is possible, that decreased expression of cav-1 α in STZ group could influence activity of eNOS and contribute to endothelial dysfunction.

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Different expression of CYP enzymes generating vasoactive arachidonic acid metabolites in animal models of glucose intolerance and diabetes

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Background and aims: Epoxyeicosatrienoic acids (EETs) are synthesized by cytochrome P450 epoxygenases from arachidonic acid (AA). In addition to their cardioprotective, antiinflammatory and hypoglycaemic effects, there is strong evidence that EETs can act as endothelium-derived hyperpolarizing factors in certain vascular beds and thus contribute to the regulation of vascular tone. An alternative route of AA metabolism by cytochrome P450 omega-hydroxylases produces proinflammatory and vasoconstrictory hydroxyeicosatetraenoic acids (HETEs). EETs are degraded by soluble epoxide hydrolase to less active dihydroxyeicosatrienoic acids (DHETs). Our aim was to investigate the expression of these enzymes in 2 animal models reflecting different stages of glucose metabolism impairment accompanied by endothelial dysfunction.

Materials and methods: In the first model, glucose intolerance (GI) was induced in 12–13 weeks old male Wistar rats (n=6–7 for each group) by postprandial streptozotocin (STZ) injections (25 mg/kg/day, i.p.) on 3 consecutive days. To induce diabetes in the second model (D), rats received STZ (30 mg/kg/day, i.p.) after overnight fasting for 3 consecutive days. Control groups (C) for each model received vehicle. 10 weeks after last dose, OGTT or preprandial blood glucose measurements were performed and we analyzed mRNA expression of chosen epoxygenases (Cyp2j4, Cyp2c23), ω -hydroxylases (Cyp4a2, Cyp4a3) and soluble epoxide hydrolase (Ephx2) in thoracic aortae by RT-qPCR. Data were statistically analyzed by Student's t-test or Mann-Whitney U test when appropriate and are represented as mean \pm SEM.

Results: In the first model we observed impaired glucose tolerance (total glucose AUC: 1835 \pm 55 vs 3079 \pm 415 mmol/l x 270min for C and GI group, respectively; $p < 0.01$), but preprandial blood glucose levels remained unchanged. In the second model, rats in diabetic group had significantly higher preprandial blood glucose (6.0 \pm 0.2 vs 29.57 \pm 0.4 mmol/l for C and D group, respectively; $p < 0.001$). The only significant change of relative mRNA expression in GI model was an upregulation of Ephx2 (1.00 \pm 0.16 vs 2.53 \pm 0.27 for C and GI group, respectively; $p < 0.05$). On the contrary, the expression of Ephx2 in D model remained at control levels, but we observed an upregulation of Cyp2j4 (1.00 \pm 0.05 vs 3.16 \pm 0.91 for C and D group, respectively; $p < 0.01$) and Cyp4a3 (1.00 \pm 0.24 vs 2.35 \pm 0.6 for C and D group, respectively; $p < 0.05$). The relative expression of Cyp2c23 and Cyp4a2 was unaltered in both models. Both models had an impaired relaxation of isolated aorta to acetylcholine.

Conclusion: Endothelial dysfunction was observed in both models, but the alterations in expression of enzymes regulating the CYP-dependent AA metabolism suggest different pathogenic mechanisms. In the glucose intolerance model, increased Ephx2 expression could enhance EETs degradation and thus contribute to endothelial dysfunction. On the other hand, in diabetes model with hyperglycaemia, the balance between the production of vasodilatory EETs and vasoconstrictory HETEs due to changes in expression of Cyp2j4 a Cyp4a. could be more important.

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Association of endothelial progenitor cells with asymmetric dimethylarginine and cardiometabolic risk factors in prediabetes

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Background and aims: It has been suggested that impaired endothelial function is present even in early states of diabetes. Endothelial Progenitor Cells (EPCs) take part in postnatal neovascularization and promote vascular homeostasis. EPCs have been considered as a potential biological marker of cardiovascular disease. Nitric oxide has been described as potential EPC mobilizing factor. Asymmetric dimethylarginine (ADMA) an endogenous inhibitor of nitric oxide synthase is a novel marker of endothelial dysfunction and atherosclerosis in humans. The aim of this study was to investigate the relation of EPC levels with several cardiovascular risk factors including ADMA among patients with prediabetes.

Materials and methods: 59 participants with newly diagnosed prediabetes and 32 controls were enrolled. Medical history, anthropometric and biochemical parameters, including traditional cardiometabolic risk factors and high sensitivity C-reactive protein (hsCRP), were obtained. Homeostasis model assessment of insulin resistance (HOMA-IR) was estimated. ADMA concentrations were determined by ELISA method. Flow cytometry identified and quantified EPCs (CD34+ KDR+ cells). Univariate and stepwise multivariate ordinal logistic regression analyses were performed using STATA 11.1 statistical software.

Results: EPCs and ADMA levels were higher in controls ($p = 0.027$) and prediabetes ($p = 0.0001$) group respectively. Univariate regression analyses performed separately for all estimated parameters in both groups. In control group EPC levels were significantly associated with: exercise ($p = 0.016$), smoking ($p = 0.002$), positive family history for diabetes ($p = 0.025$), age

($p=0.004$), c-peptide ($p<0.0001$) and ADMA ($p=0.001$). After stepwise multivariate ordinal logistic regression analysis EPC levels were significantly associated with: age (OR=0.85, 95%CI:0.77–0.94, $p=0.001$), exercise (OR=15.99, 95%CI:2.43–105.17, $p=0.004$), smoking (OR=0.01, 95%CI:0.001–0.25, $p=0.004$), and family history of diabetes (OR=0.05, 95%CI:0.01–0.36, $p=0.003$). Regarding prediabetes EPC levels were significantly associated with: BMI ($p<0.0001$), waist circumference ($p=0.007$), mean BP ($p=0.016$), c-peptide ($p=0.010$), hsCRP ($p=0.041$), statin intake ($p=0.039$) and ADMA ($p=0.004$). After stepwise multivariate ordinal logistic regression analysis EPC levels were significantly associated with BMI (OR=0.89, 95%CI:0.82–0.95, $p=0.001$) and ADMA (OR=0.64, 95%CI:0.45–0.9, $p=0.01$).

Conclusion: The ADMA-induced impaired NO bioavailability may be correlated with the reduced EPC levels and the impaired endothelial function in prediabetes. The negative and independent association of BMI with EPCs indicates a possible link between obesity and endothelial dysfunction in prediabetes, and emphasizes the importance of lifestyle modification, and especially weight management, in subjects with glucose intolerance.

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Endothelial progenitor cells show relation to asymmetric dimethyl-arginine (ADMA) levels and HbA_{1c} variability in young patients with type 1 without complications

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Background and aims: Type 1 diabetes mellitus (T1D) leads to premature cardiovascular disease. Improvement or worsening of glycemic control results in indirect proportional changes in endothelial progenitor cells (EPC) in children with T1D. Circulating EPCs play an important role in maintaining endothelial function. Asymmetric dimethylarginine (ADMA) is a competitive inhibitor of endothelial nitric oxide synthase (eNOS). ADMA is an independent marker of endothelial dysfunction and is elevated in several diseases. ADMA levels in T1D are controversial. The aim of this study is to investigate the possible association of EPCs and ADMA in T1DM.

Materials and methods: In 63 patients (29 female, 34 male) with T1D and a median age of 18.3 years (16.2;21.2) circulating EPCs were enumerated by flow cytometry. Different subsets were measured and labeled according to their antigen expression: CD34+, CD34+CD133+, CD34+CD133+CD309+, CD34+CD309+. Serum ADMA levels were measured by commercially available ELISA. HbA_{1c} variability was calculated as the coefficient of variation (CV) from HbA_{1c} values measured quarterly during one year before EPC enumeration and was available in 37 patients.

Results: There was no significant sex-dependent difference between male and female patients in age, diabetes duration (mean 11.4±3.3 years), body mass index (24.4±3.8 kg/m²) or glycemic control (mean HbA_{1c} 7.7±1.4 %), nor was there a significant correlation between glycemic control and the various EPC subsets. CD34+CD133+CD309+ positive EPC showed an inverse correlation with age ($r: -0.274$, $p=0.034$) and diabetes duration ($r: -0.261$, $p=0.044$), but not with HbA_{1c}. ADMA levels (mean 0.73±0.22 micromol/l) were neither associated with HbA_{1c} nor with age or diabetes duration, but showed a positive correlation with several of the measured EPC subsets, such as CD34+ cells ($r: 0.394$, $p=0.002$), CD34+CD133+ positive cells ($r: 0.378$, $p=0.03$) and CD34+CD133+CD309+ positive EPC ($r: 0.360$, $p=0.005$). Mean HbA_{1c} variability was 5.4±3.4% (CV). ADMA was negatively associated with HbA_{1c} variability ($r: -0.365$; $p=0.026$). Multiple linear regression analysis revealed that only serum ADMA levels remained independently and significantly ($p=0.013$) associated with CD34+CD133+CD309+ positive circulating EPC ($\beta=0.411$).

Conclusion: We show an independent and significant positive association of ADMA serum levels and circulating EPC in young patients with Type 1 diabetes mellitus without cardiovascular complications. In vitro studies demonstrated the role of ADMA as an endogenous inhibitor of mobilization, differentiation, and function of EPC. The paradoxical positive association of EPC counts and ADMA serum levels leads us to the assumption that due to oxidative stress and the consequently high ADMA levels EPC are mobilized to maintain endothelial integrity.

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The relationship between circulating irisin levels and endothelial function in obese patients

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Background and aims: Irisin is a novel hormone secreted by myocytes and could promote the browning of white adipose tissue. Some studies reported that circulating irisin levels were lower in both type 2 diabetes and obese patients compared with lean controls. Elevated NEFA levels from excess adipose tissue, which induce inflammation and oxidative stress, could account for endothelial dysfunction in obesity. It is logical to consider that irisin could ameliorate NEFA-induced endothelial dysfunction by inducing white-to-brown adipose conversion. Thus, the aim of the study was to explore the relationship between circulating irisin levels and endothelial function in obese patients.

Materials and methods: A total of 41 non-hypertensive, non-diabetic, obese patients, and 40 age- and sex-matched normal volunteers were involved in this study. Clinical characteristics, blood biochemistry, circulating irisin, and adiponectin of the subjects were measured. Endothelium-dependent vasodilation (EDV) and endothelial-independent vasodilation (EIV) in brachial artery were determined using high-resolution ultrasound.

Results: There were no significant differences in age, gender, blood pressure, total cholesterol, HDL, LDL, and fasting plasma glucose levels between two groups ($P > 0.05$). Obese patients had a higher (BMI 30.8 ± 3.8 kg/m² vs 21.3 ± 1.8 kg/m², $P < 0.01$) and larger waist circumference (WC 100.7 ± 10.47 cm vs 74.3 ± 4.6 cm, $P < 0.01$). Plasma NEFA, serum triglyceride, fasting insulin, high-sensitivity C-reactive protein (hs-CRP), and malondialdehyde (MDA) levels in obese patients were significantly higher compared with normal subjects (NEFA, 763.4 ± 150.5 umol/L vs 404.6 ± 96.1 umol/L; insulin, 18.7 ± 7.4 uU/ml vs 9.8 ± 4 uU/ml; triglyceride, 1.51 ± 0.57 mmol/L vs 0.80 ± 0.24 mmol/L; hs-CRP, 2.28 ± 0.89 mg/L vs 1.21 ± 0.32 mg/L; MDA, 4.91 ± 1.02 umol/L vs 3.70 ± 0.43 umol/L, $P < 0.01$). However, serum irisin and adiponectin levels were significantly lower in obese patients (irisin, 180.5 ± 22.4 ng/ml vs 194.8 ± 19.9 ng/ml; adiponectin, 6.04 ± 1.7 ug/ml vs 7.51 ± 1.64 ug/ml, $P < 0.05$). Endothelial function was impaired in obese patients (maximum EDV: $8.95 \pm 3.46\%$ vs $14.56 \pm 3.90\%$, $P < 0.05$). Bivariate correlation analysis revealed that circulating irisin was positively correlated with EDV ($r=0.388$, $P < 0.01$) and negatively correlated with BMI ($r=-0.281$, $P < 0.05$), WC ($r=-0.298$, $P < 0.01$), NEFA ($r=-0.289$, $P < 0.01$), hs-CRP ($r=-0.244$, $P < 0.05$), and MDA ($r=-0.258$, $P < 0.05$). EDV was positively correlated with adiponectin ($r=0.381$, $P < 0.01$) and negatively correlated with BMI ($r=-0.510$, $P < 0.01$), WC ($r=-0.484$, $P < 0.01$), triglycerides ($r=-0.412$, $P < 0.01$), NEFA ($r=-0.535$, $P < 0.01$), insulin ($r=-0.279$, $P < 0.05$), hs-CRP ($r=-0.240$, $P < 0.05$), and MDA ($r=-0.396$, $P < 0.01$). Multiple regression analysis was employed to study the association of EDV with BMI, irisin, adiponectin, NEFA, insulin, hs-CRP, and MDA. The model revealed that circulating irisin, adiponectin, NEFA, and BMI were independently associated with EDV after adjusting for covariates ($R^2=0.412$, $F=10.357$, $P=0.000$).

Conclusion: Circulating irisin level was decreased in non-hypertensive, non-diabetic, obese patients compared with normal subjects. Lower levels of irisin are independently associated with endothelial dysfunction. Therefore, irisin may be involved in the regulation of endothelial function in obesity.

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Effects of vitamin D supplement on arterial stiffness in patients with type 2 diabetes mellitus and vitamin D deficiency

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Background and aims: Vitamin D deficiency has been commonly detected in Korea and well known that deficiency is closely related with increasing risk of cardiovascular disease. Especially, the rate of Vitamin D deficiency is higher in patients with type 2 diabetes mellitus and low 25-hydroxyvitamin D [25(OH)D] level was significantly associated with increased arterial stiffness in these patients. Though there were several studies which were shown the effect of vitamin D supplement on vascular condition in vitamin D deficiency population, it is still controversy up to now. This study aimed to evaluate the

effect of vitamin D supplement on arterial stiffness in patients with type 2 diabetes and vitamin D deficiency.

Materials and methods: This study was designed as a prospective, randomized, open-label controlled trial. A total of 40 (20 men and 20 women) patients with type 2 diabetes and vitamin D deficiency, defined as serum 25(OH)D levels < 20 ng/mL, were enrolled. A randomized-study group (10 men and 10 women) was administered daily 1000IU vitamin D3 for 6 months, and control group had no interventional drug. After 6 month of intervention, authors estimated the pulse wave velocity and the aortic augmentation index as primary endpoints in both group, and statistically analyzed the variation.

Results: A mean age of patients was 56.2 years old, and mean level of 25(OH)D was 11.9 ng/mL. A 25(OH)D level of study group was significantly increased for 6 months, which were 25.3 ng/mL in study group and 9.4 ng/mL in control group ($P < 0.001$). However, there were no significant changes in terms of pulse wave velocity (-0.11 ± 0.83 vs -0.10 ± 0.26 m/sec, $P = 0.258$) and aortic augmentation index (-0.9 ± 3.7 vs $0.3 \pm 2.1\%$, $P = 0.567$). Though there is no significance, an aortic augmentation index level was tended to decrease in interventional group, compared to those of control group.

Conclusion: There were no significant changes of arterial stiffness measurement when the patients with type 2 diabetes and vitamin D deficiency were supplemented daily vitamin D, however, vitamin D level was significantly increased. This study is shown that vitamin D supplement is not clinically effective for improving arterial stiffness in patients with type 2 diabetes and vitamin D deficiency.

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Influence of postprandial hyperglycaemia on arterial function in patients with type 2 diabetes with or without albuminuria

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Background and aims: To determine whether postprandial hyperglycemia affects arterial stiffness, and whether pulse wave velocity (PWV) is a surrogate for macrovascular complications in patients with type 2 diabetes with or without albuminuria.

Materials and methods: This was a single-center, open-label study of 3 groups of males: patients with type 2 diabetes with albuminuria ($N=22$; mean \pm SD age 61 ± 5 yrs, BMI 34 ± 5 kg/m², HbA1c 62 ± 14 mmol/mol), patients with type 2 diabetes without albuminuria ($N=24$; age 64 ± 5 yrs, BMI 32 ± 6 kg/m², HbA1c 57 ± 12 mmol/mol) and healthy controls ($N=25$; age 59 ± 7 yrs, BMI 27 ± 3 kg/m², HbA1c 36 ± 3 mmol/mol). Patients were randomized to a 2-period crossover study schedule, ingesting a mixed breakfast of 500 kcal with or without insulin lispro (to induce low or high postprandial glycemia). Healthy controls were studied for 1 period with an identical breakfast. Arterial stiffness was assessed by calculating PWV and augmentation index (AIx) using applanation tonometry, and endothelial dysfunction was assessed using peripheral arterial tonometry (PAT), 30 min before breakfast and up to 240 min post-meal. These parameters were analyzed using analysis of covariance. Blood concentrations of biomarkers of inflammation, endothelial dysfunction and oxidative stress, as well as glycated proteins, were also determined.

Results: In the diabetic patients, least square mean \pm SE glucose levels at 60 min postprandial had increased by 5.3 ± 0.3 mmol/L without lispro, and by 3.3 ± 0.3 mmol/L with lispro, from 6.8 ± 0.2 mmol/L before breakfast. Before breakfast, compared with controls, aortic PWV was elevated in patients with albuminuria (mean \pm SD 11.0 ± 4.0 m/s vs. 8.6 ± 2.1 m/s, $p=0.004$), but not in patients without albuminuria (10.6 ± 4.0 m/s, $p=0.052$). Similarly, before breakfast, AIx was elevated in patients with albuminuria vs. controls ($22.6\pm 6.5\%$ vs. $17.2\pm 10.8\%$, $p=0.031$) but not in patients without albuminuria ($21\pm 6.3\%$, $p=0.111$). Adjusted for age and BMI, there were no significant differences pre-breakfast or postprandially in aortic PWV, AIx or PAT ratio, in patients vs. controls. Adjusted for age and BMI, brachial PWV was faster at 3 of 4 postprandial time-points (60, 180, 240 min; $p=0.003$ – 0.046) in patients with albuminuria vs. controls, both under high and low postprandial glycemia. No changes were observed postprandially vs. pre-breakfast in the levels of asymmetric dimethylarginine, endothelin-1, soluble vascular/intercellular cell adhesion molecule-1, superoxide dismutase, soluble extracellular domain of the receptor for advanced glycation end-products, or C-reactive protein.

Conclusion: In patients with type 2 diabetes and albuminuria, indices suggested that aortic stiffness was worse during fasting, relative to healthy controls. Also in patients with type 2 diabetes and albuminuria, brachial stiffness appeared to deteriorate when blood glucose increased postprandially by about 3–6 mmol/L.

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Endothelial glycocalyx is impaired in diabetic patients and first-degree relatives and is linked to abnormal aortic elastic properties and myocardial deformation

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Background and aims: The integrity of endothelial glycocalyx plays a vital role in vascular permeability, inflammation and elasticity. The association between damage of endothelial glycocalyx, impaired arterial elastic properties, and LV function in diabetics and first degree relatives has not been explored.

Materials and methods: In 40 untreated patients (age: 51 ± 12 years) with newly diagnosed type II diabetes, 20 first degree relatives with normal oral glucose tolerance test and 25 controls of similar age and sex and no atherosclerotic risk factors we measured: a) carotid-femoral pulse wave velocity (PWVc m/sec-Complior SP ALAM), central systolic blood pressure (cSBP -mmHg), augmentation index (AI %), reflection time (RT-ms) and diastolic reflection area (DRA) of the aortic pulse wave, an index of coronary perfusion (Arteriograph TensioMed) b) S'E' (m/sec) and E'A' of mitral annulus by Tissue Doppler c) LV longitudinal strain (GLS -%), systolic (LongSr-l/sec) and diastolic (LongSrE-l/sec) strain rate, using speckle tracking echocardiography d) perfusion boundary region (PBR- micrometers) of the sublingual arterial microvessels (ranged from 5–25 micrometers) using Sideview, Darkfield imaging (Microscan, Glycocheck). The PBR in microvessels is the cell-poor layer which results from the phase separation between the flowing red blood cells (RBC) and plasma. The PBR includes the most luminal part of glycocalyx that does allow cell penetration. Increased PBR is considered an accurate index of reduced endothelial glycocalyx thickness because of a deeper RBC penetration in the glycocalyx

Results: Compared to controls, diabetics and relatives had higher PBR (2.1 ± 0.25 vs. 2.05 ± 0.25 vs. 1.89 ± 0.1) AI (27 ± 16 vs. 24 ± 15 vs. 17 ± 14) and DRA (44 ± 12 vs. 49 ± 13 vs. 68 ± 27), ($p < 0.05$ for all comparisons). Diabetics and relatives had similar PBR, AI and DRA ($p=ns$). Compared to controls, diabetics had also higher PWV (10.9 ± 2 vs. 8.9 ± 2), cSBP (137 ± 19 vs. 116 ± 17), reduced RT (118 ± 26 vs. 151 ± 14), GLS (-16 ± 4 vs. -20 ± 3), LongSr (-0.8 ± 0.2 vs. -1.1 ± 0.3), LongSrE (0.8 ± 0.2 vs. 1.3 ± 0.5), S'E' and E'A' ($p < 0.05$ for all comparisons). Reduced endothelial glucocalyx thickness as assessed by increased PBR was related with increased PWV ($r=0.35$), reduced RT ($r=-0.42$) and DRA ($r=-0.36$) in diabetics ($p < 0.05$ for all associations). These associations were more prominent for PBR measured in the microvessels ranged from 20–25 micrometers. Increased PWV were related with reduced S' ($r=-0.48$), E' ($r=-0.63$), E'A' ($r=-0.63$), GLS ($r=0.48$), LongSr ($r=0.35$), LongSrE ($r=-0.51$) respectively ($p < 0.05$ for all associations)

Conclusion: Endothelial glucocalyx is impaired in newly diagnosed diabetics and first degree relatives and is related to abnormal aortic elastic properties leading to impaired LV longitudinal deformation in diabetics.

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PS 118 Advanced glycation

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Evaluation of a glyoxalase 1 mutant mouse

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Background and aims: Glyoxalase 1 plays an important role in the metabolism of reactive dicarbonyl metabolites, glyoxal and methylglyoxal, to less reactive products and prevention of dicarbonyl-derived advanced glycation endproduct formation. Glo1 deficient mice and transgenic mice overexpressing Glo1 provide valuable models to study control of change in extent of dicarbonyl glycation in mammalian systems. The aim of this study is to characterise the genotype and phenotype of the Lexicon Glo1 mutant mouse.

Materials and methods: The Glo1 mutant mice (+/- breeding pair), Glo1Lex were obtained from the European Mutant Mouse Archive, Heidelberg, Germany. We maintain a colony of Glo1Lex heterozygotes and sibling wild type controls. The mice were produced by Lexicon Pharmaceuticals, Inc, USA. In a C57BL/6 genetic background, mutation was produced by retroviral insertion of a DNA cassette between coding exons 1 and 2 (LEXKO-1493). Genotyping Forty-four offspring were genotyped from ear punch samples. For PCR, the three pairs of primers were used to discriminate between wild-type: heterozygote and homozygote mutant mice. Phenotyping Nineteen mice, 12 Glo1Lex (+/-) (6 male, 6 female) and 7 wild type control siblings (5 male, 2 female) were sacrificed at 7 months old, tissues (brain, heart, liver, spleen, kidney, pancreas and skeletal muscle) collected and stored at -80°C until analysis. Aliquots of tissue were homogenised and Glo1 enzymatic activity was determined in cytosolic extracts by spectrophotometric assay, protein by Western blot and mRNA by RT-PCR. Protein advanced glycation endproduct (AGE) residues of liver were analysed in 10 Glo1Lex (+/-) (5 male, 5 female) and 10 wild type control siblings (5 male, 5 female) by LC-MS/MS and normalized to the corresponding unmodified amino acid residue.

Results: In the genotyping of Glo1Lex mutant mice we found only Glo1Lex (+/-) heterozygote and wild type siblings. No homozygous Glo1Lex (-/-) mice have been born to date. No significant impairment in fertility was found for Glo1Lex (+/-) mice. The activity of Glo1 was not significantly different between wild-type controls and Glo1Lex (+/-) mice ($P < 0.05$, Mann Whitney-U). For brain, Glo1 activity (U/mg protein; median (lower - upper quartile)) was: wild type 1.77 (1.53 - 2.00), Glo1Lex (+/-) 2.05 (1.55 - 2.27). For heart, Glo1 activity was: wild type 0.95 (0.85 - 1.05), Glo1Lex (+/-) 0.98 (0.87 - 1.04). Other tissues similarly gave no significant difference in Glo1 activity between wild-type controls and Glo1Lex (+/-). The expression of Glo1, as judged by protein and mRNA, in Glo1Lex mice was not significantly different from wild-type controls. AGE residue contents of liver were not significantly different between wild type controls and Glo1Lex (+/-) mice ($P < 0.05$, Mann Whitney-U). MG-H1 residue content of liver was: wild type 0.330 (0.276 - 0.417) mmol/mol arg and Glo1Lex (+/-) 0.309 (0.276 - 0.360) mmol/mol arg. G-H1 residue content of liver was: wild type 0.125 (0.105-0.152) mmol/mol arg and Glo1Lex (+/-) 0.140 (0.112- 0.166) mmol/mol arg. Other protein glycation, oxidation and nitration adduct residue contents of liver were also not significantly different between wild type controls and Glo1Lex (+/-).

Conclusion: Glo1Lex mutant mouse appears embryonically lethal for homozygous inheritance; cf. mutant human GLO1 gene with no viable homozygous offspring. It has compensatory increased Glo1 expression with normal fertility. The mutant mouse appears to have been incorrectly genotyped in preliminary characterisation by the originator.

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1302

Knockdown of glyoxalase 1 leads to collagen overproduction in human aortic endothelial cells

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Background and aims: Chronic hyperglycaemia is an important predictor of diabetic complications and associated metabolic derangements are accompanied by accumulation of high glucose levels as well as rising methylglyoxal (MG) concentrations. MG is a highly reactive, cytotoxic carbonyl compound which is formed as a side product during glycolysis and other metabolic path-

ways. The main detoxification mechanism of MG is the glyoxalase system which consists of the enzymes glyoxalase 1 (Glo1) and glyoxalase 2 (Glo2). The aim was to analyse the impact of MG on selected proteins in human aortic endothelial cells (HAECs) under hyperglycaemia.

Materials and methods: Detection of protein and mRNA expression levels were carried out by western blot and quantitative real time PCR (qRT-PCR) analyses following siRNA mediated knockdown of Glo1 or direct stimulation experiments with exogenously added MG.

Results: Following Glo1 knockdown a significant accumulation of MG was detected by rising modifications of arginine in terms of arg-pyrimidine. Furthermore, an elevated collagen-4 and collagen-5 synthesis was detected in combination with a reduced expression of the cross-linking enzymes PLOD2 and LEPREL2. Levels of collagen-8 and collagen-18 remained unchanged in this experimental setting. In addition, an up-regulated expression of the proteoglycan metabolism associated enzymes xylosyltransferase 1 and 2 (XT1, XT2) was verified. MMP-2, TIMP-2 and TIMP-4 were elevated non-significantly. An decreased expression of Glo1 under hyperglycemia and MG stimulation (1μM) was clearly shown. MG stimulation significantly induced the expression of extracellular matrix components (collagen-4, -5, -8, -18), and the ECM-molecules TIMP-2, TIMP-4, MMP-2, TGF-β under hyperglycaemia. Comparable to the knockdown experiments the expression of proteoglycan metabolism associated enzyme XT2 was significantly elevated. Comparable to the knockdown experiments MG stimulation led to an enhanced endothelial-mesenchymal transition measured by increased expression of ACTA-2 which is in close relation to the observed overall increase in extracellular matrix components production.

Conclusion: The results of this experimental research emphasize the effect of MG on composition and stability of the extracellular matrix. This may, at least partially, explain the molecular basis of the effect of hyperglycaemia on endothelial function seen in patients with diabetes mellitus.

1303

Methylglyoxal induced protein damage in diabetes is handled by the quality control system conserved from yeast to humans

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Background and aims: Increased concentrations of reactive metabolites such as methylglyoxal (MG) are thought to be involved in the development of late diabetic complications by causing intracellular protein damage through posttranslational protein modifications. We aim to reveal cellular mechanisms and molecular pathways that are involved prevention and remission of protein damage by MG.

Materials and methods: We have established a yeast system to study protein damage and aggregate formation by increased intracellular concentrations of MG within physiological ranges. Results obtained in yeast were validated in endothelial cells in cell culture and tested in immunohistologic stains of tissue samples from patients with diabetic complications.

Results: We found that yeast forms aggregates upon increased MG concentrations. A mass spectroscopy approach to identify potential targets of MG induced protein damage revealed several molecular chaperones and metabolic enzymes. Aggregates are handled by the cellular protein quality control system. Induction of the heat shock response in general and Hsp70 in particular increased the tolerance towards increased MG levels. We next translated our findings to mammals by demonstrating that an intracellular increase of MG in mouse endothelial cells in cell culture also leads to the formation of aggregates which can be visualized with EM and are bound by Hsp70 chaperones. Pharmacological inhibition of Hsp70 rendered the cells more sensitive towards MG. Finally, staining of kidney tissue from patients with severe diabetic complications revealed drastically increased levels of Hsp70 in the glomeruli.

Conclusion: The cellular protein quality control system including Hsp70 handles MG induced protein damage from simple eukaryotes to mammals. It's up-regulation renders cells more resistant towards MG induced cell damage. These similarities between diverse species such as yeast and humans allow to exploit the high-throughput and large scale analysis tools available for simple eukaryotes such as yeast to better understand cellular responses to increased levels of reactive metabolites.

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1304

Nuclear RAGE, a regulator of DNA integrity in diabetic lung fibrosisV. Kumar¹, T. Fleming¹, E. Schleicher², H.-U. Häring², P.P. Nawroth¹;¹Internal Medicine I and Clinical Chemistry, University of Heidelberg,²Department of Internal Medicine, University of Tübingen, Germany.

Background and aims: The receptor for advanced glycation endproducts (RAGE) is a member of the immunoglobulin super-family of cell receptors, whose activation has been suggested to contribute to various pathologies. Recent studies have suggested that the localization of RAGE is not restricted to the cell-surface but also inside the nucleus interacting either directly or indirectly with DNA. However, the role that nuclear RAGE services in the nucleus remains unclear. In this study, we have investigated the ligand-independent localization of RAGE to nucleus and its role as a DNA repair protein.

Materials and methods: Pulmonary fibroblasts were isolated from the lungs of RAGE-deficient (RAGE^{-/-}) and aged-matched wild-type (C57Bl/6; male) mice. RAGE and DNA repair proteins were detected by immunofluorescence. DNA damage was assessed by single-cell gel electrophoresis (COMET) assay. Micro-irradiation of cells was performed using a FluoView1000confocal microscope, using 355nm laser. Laser settings were chosen to generate detectable double-strand breaks (DSBs) within the path of the laser, without induction of major cytotoxic effects

Results: Pulmonary phenotyping in RAGE^{-/-} mice showed that these mice had a reduced lung volume as compared to aged-matched control, consistent with a restrictive lung disease, such as fibrosis. This was confirmed by trichrome staining which showed that RAGE^{-/-} mice had a greater accumulation of collagen and other extracellular matrix. Analysis of the subcellular localization of RAGE within the lung showed that the majority of the protein was in the nucleus. The localization of RAGE to the nucleus was confirmed in vitro, using wild-type lung fibroblasts transiently transfected with GFP-tagged RAGE. It was observed that RAGE^{-/-} lung fibroblasts has a significantly altered growth rate and morphology. Further analysis showed that RAGE^{-/-} lung fibroblasts had significantly higher levels of DNA damage as compared to wild-type fibroblasts treated with and without bleomycin. The level of DNA damage could be increased in wild-type fibroblasts by transient transfection with a shRNA vector specific for RAGE. Conversely, transfection of the GFP-tagged RAGE into RAGE^{-/-} lung fibroblasts could significantly reduce the level of DNA damage by ca.80%. This would suggest, that within the context of the lung, nuclear RAGE is responsible for maintain DNA integrity. Following laser-induced DSBs, it was found that nuclear RAGE is phosphorylated by ATM, the main protein kinase sensor of DSBs, leading to its association with a variety of repair proteins, including MRN11, Rad50 and Nbs1, which are involved in the formation of the MRN complex. It was subsequently shown that post-translational modification of RAGE by methylglyoxal, a reactive metabolite elevated in diabetes, leads to loss of function with respect to DNA resectioning and binding, an effect which was not observed following oxidative modification.

Conclusion: Loss of nuclear RAGE in the lung may provide a new mechanism for the development diabetic lung fibrosis; inactivation of nuclear RAGE by reactive metabolite driven post-translational modifications would prevent the correct formation and function of the MRN complex, leading to increased DNA damage via a reduced repair capacity, analogous to the molecular phenotype observed in the RAGE^{-/-} mice.

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1305

Soluble receptor for advanced glycation end-products (sRAGE) relates to oxidative stress index in patients with diabetesJ. Škrha jr.¹, J. Soupal¹, M. Kalousova², R. Mikova¹, M. Prazny¹, J. Škrha¹;¹Third Department of Medicine, First Faculty of Medicine, Charles University in Prague, General University Hospital,²Institute of Medical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University in Prague, General University Hospital, Czech Republic.

Background and aims: Oxidative stress is one of the most important factors in the development of vascular damage in patients with diabetes. Novel findings support the idea of advanced glycation end-products involvement in the pathophysiology of diabetic angiopathy. The aim of our study was to evaluate relationship between oxidative stress and soluble receptor for advanced glycation end-products (sRAGE) in patients with diabetes.

Materials and methods: Total 121 persons were included within the study - 45 patients with Type 1 diabetes (T1DM; aged 52 ± 15 yrs), 59 patients with Type 2 diabetes (T2DM; aged 65 ± 11 yrs) and 17 healthy controls (47 ± 15 yrs). Oxidative stress was measured by Free Radical Analytical System (FRAS4; H&D, Italy) and evaluated by two blood tests: reactive oxygen metabolites test (d-ROMs) and by biological antioxidative potential test (BAP). Oxidative stress index (OSi) expressing the total oxidative status was calculated (OSi = d-ROMs/BAP). In all patients routine biochemical parameters, glycated hemoglobin (expressed in mmol/mol acc. EFCC), sRAGE, cell adhesion molecules (VCAM, ICAM), von Willebrand factor, (micro)albuminuria (expressed as albumin/creatinine ratio UACR), and anthropometrical data were measured.

Results: Oxidative stress index was similar between groups (T1DM: 0.25 [0.13 - 0.47], T2DM: 0.25 [0.13 - 0.42], controls 0.24 [0.18 - 0.34]; NS) and also sRAGE did not differ significantly (T1DM: 1352 ± 514, T2DM: 1179 ± 814, controls: 1177 ± 564 ng/l, NS). Significantly lower d-ROMs were observed in patients with T1DM and T2DM without albuminuria (UACR<2.5 g/mol creatinine) as compared to patients with albuminuria (368 ± 105 vs. 432 ± 134 U; p=0.009). Significant positive relationship of OSi and sRAGE was found both in T1DM (r=0.39; p<0.01) and T2DM (r=0.33; p<0.02), and similarly of OSi and VCAM (T1DM: r=0.47, p<0.005; T2DM: r=0.35, p<0.05).

Conclusion: This is the first study describing significant relationship between oxidative stress expressed by oxidative stress index and sRAGE concentration in patients with diabetes. Similar relationship was observed between OSi and marker of vascular activation - adhesion molecule VCAM. Oxidative stress, apart from direct effect, likely participate in the development of endothelial activation also by other mechanisms - e.g. RAGE activation. Though, more studies will be necessary for better understanding of these processes.

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1306

One-compartment kinetic model predicts dicarbonyl modification influence half-life and functionality of HDL in high risk CVD groupN. Rabbani¹, L. Godfrey¹, N. Yamada-Fowler¹, J. Smith², P.J. Thornalley¹;¹Clinical Sciences Research Institute, University of Warwick,²Bruker UK Ltd, Coventry, UK.

Background and aims: Decreased plasma concentration and anti-atherogenic properties of high density lipoprotein cholesterol (HDL-C) is implicated in increased CVD risk. The cause is unknown and under intense investigation. The extent of modification of HDL by methylglyoxal, a reactive dicarbonyl metabolite, in patients with diabetes and related functional effects is also unknown. Dicarbonyl modification of HDL may be linked to the reported 20% and 37% decrease in half-life of HDL in type 2 diabetes and renal failure. The aim of this study was to: (i) to construct a one-compartment model of HDL influx and clearance from plasma in human subjects, and (ii) from the effect of minimal modification of HDL by methylglyoxal on HDL plasma clearance, predict how dicarbonyl glycation influences plasma HDL concentration in patients with diabetes, renal failure and the ageing population.

Materials and methods: The extent of dicarbonyl glycation of HDL - modification by methylglyoxal and related metabolites glyoxal and 3-deoxyglucosone - was assessed by quantitation of dicarbonyl-derived AGEs in HDL protein. We constructed a one-compartment model of HDL release into and clearance from plasma assuming that dicarbonyl-modified HDL is preferentially cleared. We measured the change in clearance of HDL when modified minimally by methylglyoxal in rats. The mathematical model of HDL influx and clearance from plasma in human subjects was defined by published estimates of values of apolipoprotein (ApoA1) synthesis (14 mg kg⁻¹day⁻¹) and HDL half-life (4.47 days), and validated experimentally by measurement of plasma HDL and methylglyoxal-modified HDL in human subjects. HDL kinetics were computed for 2 - 4 fold increase in dicarbonyl concentration typical of old-age, diabetes and renal failure using the COPASI programme.

Results: Dicarbonyl-modified HDL accounted for ca. 2.6% HDL in healthy people. Experimental studies in rats showed that clearance of HDL from plasma increased 2-fold when modified minimally by methylglyoxal. Input of a 2 - 4 fold increase in plasma dicarbonyl concentration into the one compartment model of plasma HDL predicted a 2 - 6% decrease in plasma total HDL. ApoA1 concentration correlates strongly with HDL-C (r = 0.83) and so this translates to a predicted ca. 3 - 9% increased risk of CVD. The model also predicted a greater decrease in functional HDL (4.5 - 12.5%). Suggested validity of the model came from experimental confirmation of the predicted negative association of plasma HDL-C to the level of methylglyoxal modified

HDL in healthy people. From the correlation coefficient, $r = -0.42$, $P < 0.05$ ($n = 22$, Pearson), glycation of HDL by methylglyoxal accounts for 18% of the variation in HDL-C.

Conclusion: The kinetic model predicted dicarbonyl modification contributes significantly to decreased half-life and dysfunction of HDL, and through this to increased CVD risk in high risk populations such as elderly and patients with type 2 diabetes and renal failure. Therapeutic interventions to counter dicarbonyl glycation of HDL may decrease risk of CVD.

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1307

Protection from diabetes-induced atherosclerosis and renal disease by octyl-D-carnosine: evidence for a role of AGEs in hyperglycaemic memory

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Background and aims: The advanced glycation end-products (AGEs) are involved in the pathogenesis of vascular and renal complications of diabetes and are considered as a putative mechanism for “hyperglycaemic memory”. Octyl-D-carnosine (ODC) was shown to attenuate AGE formation as well as vascular and renal injury induced by high fat diet in ApoE null mice. This study was aimed at verifying the protective effect of ODC in atherosclerosis and renal disease induced by experimental diabetes and the role of AGEs in “hyperglycaemic memory”.

Materials and methods: To this end, ApoE null mice were rendered diabetic by multiple injections of streptozotocin and divided in 4 groups: untreated (DIAB) or treated with ODC for 20 weeks (ODC-Extended) or for first 11 weeks only, starting immediately after diabetes induction (ODC-Early) or at week 9 (ODC-Late). Non-diabetic ApoE null mice served as controls. Metabolic parameters were assessed using standard methods. Aortic and renal lesions were evaluated by morphometry. Protein and gene expression of relevant disease markers were assessed by immunohistochemistry and RT-PCR, respectively.

Results: Treatment ODC-Extended was effective in reducing the size of atherosclerotic lesions ($48,496 \pm 5,501$ vs. $90,545 \pm 4,267 \mu\text{m}^2$), and plaque content of macrophages (11.9 ± 3.2 vs. 39.9 ± 3.3 % lesion area, and 1.46 ± 0.36 vs. 3.4 ± 0.69 UA), MCP-1 (1.74 ± 0.58 vs. 3.61 ± 0.63 UA), RAGE (10.4 ± 1.6 vs. 37.3 ± 3.3 % lesion area, and 1.12 ± 0.31 vs. 2.41 ± 0.44 UA), N^ε-carboxymethyllysine (CML, 16.6 ± 3.1 vs. 64.0 ± 6.2 % lesion area), nitrotyrosine (11.4 ± 2.7 vs. 37.1 ± 4.5 % lesion area), and apoptotic cells (24.8 ± 4.4 vs. 88.8 ± 6.8 n/mm²). Conversely, plaque content of collagen (42.3 ± 3.3 vs. 26.4 ± 2.5 % lesion area) and smooth muscle cells (13.4 ± 2.1 vs. 4.9 ± 1.1 % lesion area) was increased, as compared with DIAB. ODC treatment for 11 weeks afforded partial protection from atherosclerotic lesions, which however was significantly higher in ODC-Early than in ODC-Late for most of the above parameters. In fact, ODC-Early plaques were smaller as compared with ODC-Late plaques and showed reduced content of CML, which was associated with less inflammation and oxidative stress markers, smaller necrotic cores, increased deposition of collagen and thicker fibrous caps. In addition to quantitative differences, also qualitative differences were observed. In particular, immunohistochemistry staining for CML, macrophage, apoptosis and oxidative stress markers was restricted to the luminal side of the ODC-Early plaques, at variance with what observed in plaques from ODC-Late and untreated DIAB mice, which covered the entire depth of the atherosclerotic intima. Also renal disease was attenuated in ODC-Extended than in DIAB and partial protection was observed in mice treated for 11 weeks, with no significant difference between ODC-Early and ODC-Late.

Conclusion: These data show that therapy with ODC protects from vascular and renal disease induced by experimental diabetes and early treatment is more effective than late treatment on atherosclerosis, thus suggesting that inhibition of AGE formation and accumulation within early plaques might slow down post-treatment atherosclerosis progression and favour development of more stable lesions. This is consistent with the hypothesis that AGEs could represent the underlying mechanism responsible for the “hyperglycaemic memory”.

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PS 119 Metabolism and complications

1308

Glucagon-like peptide-1 reduces intestinal lipoprotein production by peripheral pathways and central melanocortin-4 receptor signalling

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Background and aims: The intestinal overproduction of chylomicron particles is a common feature of insulin resistance and the metabolic syndrome, resulting in postprandial dyslipidemia and heightened levels of atherogenic particle remnants. Recently, the anti-diabetic hormone glucagon-like peptide-1 (GLP-1) has gained attention not only as an insulin secretagogue, but also as a modulator of intestinal lipoprotein metabolism in the periphery. GLP-1 can additionally be produced within the brain to regulate neuronal activity in regions like the arcuate nucleus of the hypothalamus, and through sympathetic pathways GLP-1 has been shown to modulate peripheral lipid metabolism. Here we investigated the involvement of both peripheral and central GLP-1 in regulating intestinal chylomicron production.

Materials and methods: Healthy hamsters received an oral gavage of olive oil followed by an acute intraperitoneal (IP) or intracerebroventricular (ICV) injection of the GLP-1 receptor (GLP-1R) agonist exendin-4 (5nmol/kg and 250ng, respectively) or vehicle. The postprandial production of triglyceride (TG)-rich lipoproteins (TRL) was then assessed over 6h by measuring levels of TRL-TG and -apolipoprotein B48 (apoB48). This study was repeated with 3μg ICV MK-0626, an inhibitor of endogenous GLP-1 degradation, in the presence or absence of the GLP-1R antagonist exendin9-39. To characterize the central pathways of GLP-1 action, the effects of ICV exendin-4 on TRL production were assessed during ICV melanocortin-4 receptor (MC4R) antagonism and in the presence or absence of α and β adrenergic receptor blockers given intravenously.

Results: Both IP and ICV exendin-4 treatment induced prolonged reductions in the rate of TRL-TG accumulation (IP 72% $p < 0.05$ and ICV 56.5% $p < 0.01$). A similar trend was seen in apoB48 accumulation. The effects of central exendin-4 were mirrored by ICV administration of MK-0626 and negated by ICV pre-treatment with the GLP-1R antagonist exendin9-39. This indicates that GLP-1 produced in the brain can act on central GLP-1Rs to modulate chylomicron production in the periphery. In accordance with satiety studies supporting the notion that central GLP-1 may signal through the MC4R system, central MC4R antagonism prevented ICV exendin-4 from lowering TRL-TG levels. An infusion of adrenergic receptor antagonists also prevented the reduction in TRL-TG levels seen with exendin-4 treatment alone, suggesting that the GLP-1-sensitive brain-gut axis modulating chylomicron production is likely via sympathetic pathways. Finally, to study the interplay between central and peripheral pathways of GLP-1R signaling, exendin-4 was given IP while exendin9-39 was given ICV and vice-versa. IP exendin-4 maintained its ability to diminish TRL-TG levels during central antagonism; however, IP exendin9-39 prevented the action of ICV exendin-4. This suggests that central stimulation may potentiate peripheral GLP-1Rs, and that peripheral GLP-1Rs can act independently of central signaling to modulate intestinal lipoprotein metabolism.

Conclusion: Our results demonstrate an important role for central GLP-1R signaling in modulating chylomicron production, which has critical implications for diabetic dyslipidemia. In line with previous studies showing that exendin-4 activates neurons in the arcuate nucleus, this brain-gut axis appears to involve MC4Rs and sympathetic outflow to the periphery.

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1309

Serum paraoxonase-1 activity is more closely related to HDL particle concentration and large HDL particles than to HDL cholesterol in type 2 diabetic and non-diabetic subjectsR.P.F. Dullaart¹, J.D. Otvos², R.W. James³;¹Endocrinology, University Medical Center, Groningen, Netherlands,²LipoScience Inc., Raleigh, USA, ³Internal Medicine, Division of Endocrinology, Diabetology, Hypertension and Nutrition, University Hospital Geneva, Switzerland.

Background and aims: We determined relationships of the anti-oxidative enzyme, paraoxonase-1 (PON-1), with high density lipoprotein (HDL) sub-fractions, and tested whether these relationships are stronger than those with HDL cholesterol and apolipoprotein A-I (apoA-I) in subjects with and without type 2 diabetes mellitus (T2DM).

Materials and methods: Serum PON-1 (arylesterase activity) and HDL sub-fractions (nuclear magnetic resonance spectroscopy) were determined in 67 T2DM patients and in 56 non-diabetic subjects.

Results: PON-1 activity, HDL cholesterol and apoA-I were decreased in T2DM (all $p < 0.05$). The HDL particle concentration was unaltered, but large HDL particles, medium HDL particles and HDL particle size were decreased, whereas small HDL particles were increased in T2DM (all $p < 0.05$). PON-1 was more closely related to HDL cholesterol than to apoA-I ($p = 0.001$). In turn, the positive relationship of PON-1 with the HDL particle concentration and with large HDL particles was stronger than that with HDL cholesterol (both $p < 0.01$). The inverse relationship of PON-1 with T2DM was only modestly attenuated by HDL cholesterol or HDL particle characteristics. Multi-variable linear regression analyses demonstrating relationships of serum paraoxonase-1 activity with mutually adjusted high density lipoprotein (HDL) variables and apolipoprotein A-I (apoA-I) in 63 subjects with Type 2 diabetes mellitus (T2DM) and in 56 non-diabetic subjects. HDL variables are given in standard deviation scores (Z-scores). Independent statistical determined as assessed by subsequent backward elimination are shown in bold print. β : standardized regression coefficient. Model 1: HDL cholesterol + apoA-I; model 2: HDL cholesterol + HDL particle concentration; model 3: HDL cholesterol + large HDL particles.

Conclusion: PON-1 activity is more closely related to the HDL particle concentration or large HDL particles than to HDL cholesterol. Impaired PON-1 activity in T2DM is not to a considerable extent explained by altered HDL subfraction levels.

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1310

HDL and blood glucose correlations in patients with familial hypercholesterolaemiaM. Walus-Miarka¹, B. Idzior-Walus¹, M. Małeck¹, P. Miarka², E. Wozniakiewicz²;¹Metabolic Diseases, CMUJ, ²Nephrology, CMUJ, Krakow, Poland.

Background and aims: Familial hypercholesterolemia is characterized by markedly increased LDL-cholesterol concentration due to mutation in LDL-receptor, apo B 100 and PCSK9 genes and, in consequence, premature coronary artery disease. Typically HDL-C and triglyceride concentrations are within normal limits. Accumulating data suggest that cholesterol homeostasis is a major regulator of beta cell function. Intra-cellular cholesterol accumulation leads to islet dysfunction and impaired insulin secretion. There is also evidence suggesting that the different lipoprotein classes have varying effects on beta cell apoptosis and proliferation. The aim of our study was to assess blood glucose correlates in patients with familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCH) and healthy controls (C).

Materials and methods: Material included patients from outpatient lipid clinic, referred for treatment of resistant hyperlipidemia. Familial hypercholesterolemia was diagnosed according to Simon Broome register criteria. In each patient standardized questionnaire, anthropometric examinations and lab test were performed. Serum lipids and glucose were examined in fasting state by enzymatic methods, using Roche reagents.

Results: We examined 241 patients with FH, 124 patients with FCH and 45 controls. Mean age and BMI values in FH, FCH and C groups were as follows: 41.1 \pm 18.4, 41.8 \pm 15.0 and 32.5 \pm 15 years; 24.7 \pm 5, 26 \pm 4.4 and 22.4 \pm 4.4 kg/m². Mean values of serum concentrations of LDL-cholesterol in FH, FCH and controls were 4.6 \pm 1.6, 3.5 \pm 0.7 and 2.5 \pm 0.6, while of HDL-cholesterol; 1.6 \pm 2.2, 1.5 \pm 0.4, and 1.5 \pm 0.5 mmol/l respectively. In

FH patients serum glucose concentration significantly correlated with age and obesity parameters (BMI and waist circumference) and blood pressure values. We also found significant correlation between serum glucose and creatinine ($r = 0.15$, $p = 0.02$), GGTP ($r = 0.13$, $p < 0.05$) and a strong negative correlation with HDL-cholesterol ($r = -0.26$, $p < 0.001$). In FCH group apart from correlation with age, obesity indices, blood pressure and creatinine, significant association between glucose and serum triglyceride, insulin resistance syndrome component, was found ($r = 0.18$, $p < 0.05$). In healthy persons only significant positive correlations between serum glucose and CRP and negative with serum creatinine were observed. Multiple regression analysis with serum glucose as dependent variable revealed that in FH patients serum HDL-C concentration ($p = 0.0009$) and waist/hip ratio ($p = 0.014$) were significant associates. In statin treated FH patients serum glucose concentrations were associated with the same variables as in the whole group of patients with exception of lack of correlation with HDL-C, similarly like in healthy controls, and the presence of negative correlation between LDL-C ($r = -0.18$, $p < 0.05$). In FH patients not taking statins only the negative correlation between HDL-C and serum glucose was observed.

Conclusion: The results of the study suggest that HDL-C and WHR are significant correlates of blood glucose levels in FH patients, while HDL-C is associated with blood glucose levels only in patients not treated with statin. These results might be of importance in development of carbohydrate metabolism disturbances in statin treated FH patients.

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1311

Diet induced fat storage in the ApoE knockout rat: a novel animal modelS. Skovso¹, I. Rune^{1,2}, X.A. Wolf², B. Rolin²;¹University of Copenhagen, ²Translational Pharmacology, Novo Nordisk A/S, Måløv, Denmark.

Background and aims: Apolipoprotein E (ApoE) is highly expressed in adipose tissue and plays a pivotal role in lipoprotein and triglyceride metabolism in adipocytes. The role of ApoE in site-specific fat storage remains poorly understood. The contribution of ApoE in adipocyte triglyceride accumulation has previously been described in ApoE knockout mice, but not in ApoE-deficient rats. Moreover, the effect of dietary gluten as a potential accelerator of site specific fat accumulation has not been thoroughly examined. Fat distribution and cardiovascular risk are highly associated. Thus, we investigated site specific fat storage in rats with systemic knockout of the ApoE gene, fed either a cholesterol enriched Western Diet or a normal Low Fat diet in combination with or without gluten.

Materials and methods: ApoE knockout rats were fed either: 1) a Western Diet (WD) high in cholesterol, fat and sucrose; 2) a WD with gluten (WD+G); 3) a Low Fat diet (LF); or 4) LF and gluten (LF+G) from 10 days of age. At 20 weeks of age body weight, whole body fat, visceral fat and subcutaneous fat were measured. Fat distribution was measured by MRI and CT scanning

Results: We compared rats fed either WD or LF diets in combination with or without gluten at 20 weeks of age. The greatest differences were observed between rats fed WD+G diet or LF+G diet. When compared to LF+G fed rats, WD+G fed rats had significantly higher body weight (352 \pm 14 g versus 293 \pm 11 g; $p < 0.001$), whole body fat/body weight ratios (0.26 \pm 0.081 versus 0.17 \pm 0.010; $p < 0.001$), visceral fat (13.4 \pm 0.9 g versus 7.1 \pm 0.7 g; $p < 0.01$), and subcutaneous fat (5.1 \pm 0.3 g versus 2.5 \pm 0.4 g; $p < 0.001$). No significant differences were observed between LF and WD, between WD and WD+G or between LF and LF+G for any of the above mentioned parameters. However, the visceral fat was significantly increased in WD+G fed rats, when compared to WD fed rats (13.4 \pm 0.9 g versus 10.8 \pm 0.7 g; $p < 0.05$). Finally, the liver density, an inverse measure of liver fat, was observed to be significantly lower in the WD+G fed rats, when compared to the WD rats (-46.1 \pm 3.6 Hounsfield Units (HU) versus -19.9 \pm 5.1 HU, $p < 0.01$) at week 20.

Conclusion: Our data suggest that WD in combination with gluten, but not alone, increases deposition of fat in the visceral compartment rather than the subcutaneous compartment in ApoE knock out rats when the different diets are introduced from 10 days of age. The differences observed in body weight, whole fat and the site of fat deposition between the group fed WD combined with gluten and the group fed LF diet combined with gluten also suggest that it is the interaction between gluten and the cholesterol-enriched WD, rather than gluten per se, that promotes the early signs of the metabolic syndrome.

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The synergistic effect of two mtDNA point mutations in complexes of the respiratory chain promote age-dependent hyperglycaemia and mitochondrial ROS production

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Background and aims: Mutations in complexes of the respiratory chain, which are encoded in the mitochondrial genome (mtDNA), can mediate an increase in production of reactive oxygen species (ROS). Thus, mtDNA mutations are proposed to evoke mitochondrial dysfunction and to contribute to the pathogenesis of type 2 diabetes mellitus. In this study we investigated ROS production and the expression of antioxidative enzymes in liver and muscle from conplastic mice carrying a mtDNA point mutation in the cytochrome c oxidase (complex IV) or a combined mutation in the cytochrome c oxidase (complex IV) and the NADH dehydrogenase (complex I).

Materials and methods: Blood glucose was measured in the conplastic mouse strains C57BL/6NTac-mtBPL/1J (NADH dehydrogenase mutation und cytochrome c oxidase mutation, mtBPL), C57BL/6NTac-mtNOD/LtJ (cytochrome c oxidase mutation, mtNOD) and C57BL/6NTac-mtAKR/J (control; mtAKR). For analysis of mitochondrial ROS production MitoSox was injected in living animals. Thereafter mice were killed and tissues were analyzed for accumulated ROS by fluorescence microscopy. Gene expression of the antioxidative enzymes catalase, SOD1, SOD2 was investigated by quantitative PCR analyses.

Results: At the age of 6 months mtBPL mice (7.5 mmol/l) showed significantly higher blood glucose levels compared to mtNOD mice (6.1 mmol/l) and mtAKR control mice (6.2 mmol/l). In liver and muscle of mtNOD and mtAKR mice an age-dependent increase in ROS was observed between month 3 and 12. In contrast, mtBPL mice showed a significant 20-fold peak increase in ROS at the age of 6 months, which normalized at the age of 12 month. At this time point the ROS level in muscle and live was comparable to mtAKR control mice. In dependence of age mtNOD mice showed a comparable or higher expression of antioxidative enzymes than mtAKR control mice, whereas in mtBPL mice the expression of all antioxidative enzymes and in particular the expression of catalase was significantly reduced.

Conclusion: The coincidence of mtDNA point mutations in the cytochrome c oxidase and NADH dehydrogenase resulted in higher blood glucose levels in 6 month old mtBPL mice. At this age mtBPL mice showed a significant increase of ROS production in liver and muscle. Thus, mtDNA mutations confer high ROS production in liver during adolescence with inadequate adaptation of the antioxidative system. This scenario could be a pathogenic trigger for insulin resistance with progressive ageing and contribute to the development of type 2 diabetes mellitus.

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Streptozotocin-induced diabetes does not affect mitochondrial structure and function

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Background and aims: A central mechanism underlying the development and progression of late diabetic complications is increased production of reactive oxygen species (ROS). Mitochondria, and specifically the electron transport chain (ETC), are considered to be the main source for intracellular ROS. During hyperglycemia, ROS production is increased leading to changes in the function of mitochondria including decreased activity of the ETC complexes, reduced ATP production, as well as changes in morphology. Unpublished data from our research group have shown discrepancies between hyperglycemic conditions and elevated ROS levels. To estimate whether mitochondria dysfunction is associated with diabetes, the structure and functional properties of mitochondria were studied in the organs of streptozotocin-induced diabetic mice, using high-through put screening assays.

Materials and methods: Diabetes was induced in healthy, wild-type mice (C57BL/6; male), by low-dose streptozotocin treatment. After four months, the hearts, kidneys, and livers from control and diabetic mice were collected, and mitochondria isolated by differential centrifugation. Purity of the isolated mitochondria was determined by electron microscopy (EM) and FACS. Morphology of the mitochondria within the tissue was also assessed by EM. The abundance of ETC complexes was analyzed by western blot (WB) and

the activity of the complexes was tested using specific activity assays. Total oxygen consumption, ATP, and ROS production were analyzed during state 3 and state 4 respiration.

Results: After four months of diabetes, both blood glucose (158.8 ± 4.1 mg/dl vs 467.8 ± 75.7 mg/dl, P-value = 0.0002) and HbA1c (2.9 ± 0.1 % vs 9.5 ± 0.4 %, P-value < 0.0001) were significantly increased in the diabetic group, and body weight was significantly decreased (36.1 ± 1.2 mg vs 23.8 ± 2.0 mg, P-value < 0.0001). The mitochondria fraction isolated from the organs of each group had a purity of more than 95 %. No gross morphological changes between control and diabetic groups were observed in either the isolated mitochondria or within the tissue. No differences were observed with respect to the abundance of the ETC complexes, ATP, and ROS production. However, during state 3 respiration, both heart- and liver-derived mitochondria from diabetic mice showed increased oxygen consumption. During state 4 respiration, oxygen consumption was increased in liver-derived mitochondria from diabetic mice. No changes could be observed at either state 3 or 4 for kidney-derived mitochondria.

Conclusion: This study describes a methodology not only for the isolation of high purity mitochondria from tissue, but also high-through put screening of mitochondrial function. Utilizing this methodology, it was found that at four months of streptozotocin-induced diabetes, there were little or no changes in the structure and function of mitochondria. Additional studies are required to determine whether mitochondria dysfunction is observed at later stages of diabetes and is associated with increased ROS production.

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Pyruvate kinase isoenzyme M2 shows increased expression in diabetes

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Background and aims: Pyruvate kinase (PK) catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, generating ATP as the last step of glycolysis. PK is expressed in 3 different isoforms named M1, M2 and LR. PKM1 and PKM2 show different activity levels, with PKM2 being highly expressed in cancer where enables cells to use aerobic glycolysis instead of oxidative phosphorylation. mRNA expression of the different isoforms (M1-M2) was investigated using specific primers and siRNAs in control and diabetic material from mouse and human in order to investigate whether PKM isoforms show differential expression in diabetes and how this balance affects late diabetic complications.

Materials and methods: Hearts, kidneys, sciatic nerves (SN) and dorsal root ganglia (DRG) derived from 3-6 months control and diabetic mice and human peripheral blood mononuclear cells (pBMCs) derived from healthy aged matched control and type 2 diabetic individuals were used for RNA extraction, cDNA preparation and subsequent real-time PCR using specific primers for the different PKM isoforms (M1-M2). Additionally, in vitro experiments were performed by the use of siRNA specific for either both or M2 PK isoform in order to measure metabolic dysfunction with respect to glycolysis and the electron transport chain.

Results: PKM isoforms show differential expression in diabetic tissues and cells in compare to the respective controls. PK M1/M2 ratio is significantly decreased in hearts, kidneys, SN and DRG derived from diabetic mice in compare to age matched control mice. The same ratio is also decreased in human pBMCs derived from diabetic individuals in compare to control ones with a positive correlation being found between the levels of PKM2 isoform and the neuropathy symptom score (NSS) in the diabetic group.

Conclusion: PK as the last enzyme of glycolysis plays an important role in cell metabolism. This study reveals a differential expression of the two major PK isoforms (M1-M2) in diabetes. The increased PKM2 expression in diabetic tissue can be correlated with the ability of cells to use aerobic glycolysis instead of oxidative phosphorylation. These findings reveal new aspects in development of late diabetic complications, thereby providing new opportunities for therapeutic interventions.

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PS 120 Animal models of complications I

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Treatment with divalent copper chelation reverses defective myocellular copper transport in the heart of diabetic rat

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Background and aims: Heart disease is the leading cause of death in diabetic patients, and defective copper metabolism could play important roles in the pathogenesis of diabetic cardiomyopathy. This study was to determine how myocardial copper status and myocellular copper chaperon proteins become impaired by diabetes, and how they respond to treatment with the Cu(II)-selective chelator triethylenetetramine (TETA).

Materials and methods: Experiments were performed using streptozotocin (STZ)-induced diabetic rats with and without TETA treatment. Cardiac function were analysed in isolated, *ex-vivo* perfused working hearts and myocardial copper content measured using particle-induced x-ray emission spectroscopy coupled with Rutherford backscattering spectrometry. The expression (mRNA and proteins) and/or activity of key protein components involved in copper binding and transport were analysed using combination of RT-qPCR, western blotting, immunofluorescence staining and enzyme activity assays.

Results: Left-ventricular (LV) copper levels and LV function were markedly deficient (~50%, $p < 0.01$) in rats following 16-weeks' diabetes in STZ-rats, but both were unexpectedly normalized after 8-weeks' treatment with TETA. Localized myocardial copper deficiency was accompanied by decreased expression (~40–50%, $p < 0.05$) and increased polymerization of the copper-responsive transition-metal-binding metallothionein proteins (MT1/MT2), consistent with impaired anti-oxidant defences and elevated susceptibility to pro-oxidant stress. Levels of the high-affinity copper transporter-1 (CTR1) were depressed (~30%, $p < 0.01$) in diabetes, consistent with impaired membrane copper uptake, and were not modified by TETA which, contrastingly, renormalized myocardial copper and increased levels (~1.5 fold, $p < 0.05$) and cell-membrane localization of the low-affinity copper transporter-2 (CTR2). Diabetes also lowered indexes (~30%, $p < 0.01$) of intracellular copper delivery via the copper chaperone for superoxide dismutase (CCS) to its target cuproenzyme, superoxide dismutase-1 (SOD1): this pathway was restored by TETA treatment, which normalized SOD1 activity with consequent bolstering of anti-oxidant defences. Furthermore, diabetes depressed levels (~20%, $p < 0.01$) of additional intracellular copper-transporting proteins, including antioxidant-protein-1 (ATOX1) and Cu(II)-transporting-ATPase-2 (ATP7B), whereas TETA elevated copper-transporting-ATPase-1 (ATP7A) (~20%, $p < 0.01$).

Conclusion: Myocardial copper deficiency and defective cellular copper transport/trafficking are revealed as key molecular defects underlying LV impairment in diabetes, and TETA-mediated restoration of copper regulation provides a potential new class of therapeutic molecules for diabetic heart disease.

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Exendin-4 counteracts palmitate-induced autophagy in human cardiac progenitor cells by inhibiting p38 MAPK phosphorylation and de novo production of ceramide

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Background and aims: Increased autophagy of cardiomyocytes has been proposed as a mechanism of myocardial damage and dysfunction. Glucagon-like peptide-1 (GLP-1) and GLP-1 analogs exert protective effects on cardiac cells. In this study, the effects of exendin-4 (Ex-4) on lipotoxicity-induced autophagy were investigated in human cardiac progenitor cells (hCPC).

Materials and methods: hCPCs were isolated from right auricle biopsies of patients undergoing elective heart surgery and exposed to palmitate (0.25 mM up to 16 h). Autophagy was evidenced by monodansyl cadaverine and autophagolysosome labeling, and by immunoblotting of microtubule-associated protein 1 light chain 3 (LC3)-II and beclin1. p38 expression and phosphorylation was detected by immunoblotting. The expression of ceramide synthase 5 (CerS5) was studied by RT-PCR and immunoblotting. Intracellular ceramide content was evidenced by immunofluorescence.

Results: Palmitate induced an increased autophagy in hCPC, evidenced by monodansyl cadaverine and autophagolysosome labeling, and confirmed by increased levels of microtubule-associated protein 1 light chain 3 (LC3)-II and beclin1, respectively ($p < 0.05$). Palmitate also stimulated p38 MAPK phosphorylation ($p < 0.05$), and pretreatment with the p38 MAPK inhibitors SB203580 and SB202190 significantly inhibited palmitate-induced autophagy. In palmitate-exposed hCPC, intracellular ceramide content, evaluated by immunofluorescence, was concomitantly augmented, in parallel with increased expression of ceramide synthase 5 (CerS5), a critical enzyme in ceramide generation. Co-incubation of hCPCs with fumonisins-B1, a specific CerS5 inhibitor, partially prevented palmitate-induced autophagy. However, the inhibition of CerS5 with a specific siRNA did not prevent p38 MAPK phosphorylation induced by palmitate. When hCPC were pretreated with Ex-4, the palmitate-induced increase in LC3-II and beclin1 and p38 MAPK phosphorylation were abrogated ($p < 0.05$), and so was the increase in CerS5 and ceramide levels ($p < 0.05$).

Conclusion: Palmitate-induced hCPC autophagy is mediated both by p38 MAPK activation and de novo ceramide synthesis in parallel, and the GLP-1 analog Ex-4 can counteract these responses. GLP-1 mimetics may thus protect the myocardium from lipotoxic damage in type 2 diabetic and/or obese subjects with elevated free fatty acid levels.

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Cardiac immuno-inflammatory response to dietary sodium restriction in rats with insulin resistance and hypertension

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Background and aims: Controversial influence of dietary sodium restriction was reported in rats with insulin resistance. We have recently observed that low sodium (LS) diet prevents renal damages in rats fed high fructose. In the present study, we aimed at verify whether LS diet may also prevent fructose-induced modifications in the heart, even in presence of a moderate arterial hypertension. In addition, the involvement of the immuno-inflammatory response in the beneficial effect of a low sodium diet was assessed in cardiac tissue.

Materials and methods: At the age of 8 weeks, Sprague-Dawley rats received three different regimens for 8 weeks. Low sodium (LS, <0.01% NaCl) or normal sodium (NS, 0.65% NaCl) diets were proposed to rats made insulin resistant with high fructose consumption (60%, $n = 20$) and hypertensive by chronic infusion of Angiotensin II (AngII, 200 ng.kg⁻¹.min⁻¹, sc, $n = 20$). After 4 weeks of the fructose diet, AngII was infused or not during an additional 4-week period on fructose. A regular rat chow with NS content was given to control rats. Tail-cuff pressure (TCP) was measured before and after AngII. Heart weight index (HWI, mg HW / g BW) as well as the cardiomyocytes size (histology) were determined at the end of study. Insulin resistance was determined through the use of an insulin tolerance test (ITT, 0.6 UI/kg, ip). Cardiac expression of various genes was assessed by real-time PCR (qPCR). Presence of monocytes/macrophages was evidenced by immunostaining and expression of CD68. Transcription factor, NF- κ Bp65, and the presence of CD4+ and CD8+ T cells or B cells (CD20) were evaluated by qPCR.

Results: As expected, AngII induced a clear rise in TCP in rats fed NS fructose diet ($40 \pm 5\%$ from basal value of 139 ± 2 mmHg, $P < 0.01$). TCP rose to a lesser extent ($20 \pm 4\%$, $P < 0.05$) in rats fed LS fructose diet. The response to ITT was similar in NS and LS rats fed high fructose and infused with AngII. When compared to standard rats, fructose induced a cardiac hypertrophy with a HWI of 2.80 ± 0.05 vs 2.44 ± 0.07 mg/g, $P < 0.05$. HWI was lower in rats fed the LS- than the NS-fructose diet (~8% vs 15%). HWI further increased in AngII-infused rats on both diets (HWI of 3.01 ± 0.28 in NS and 2.89 ± 0.07 mg/g in LS); yet the rise was less marked in LS- than NS-fed rats (~9 vs 23% compared to control group). The rise in cardiomyocytes size was prevented by the LS diet. In the heart, macrophages infiltration (CD68 staining and expression) during fructose feeding was reduced by the LS diet in absence ($9.5 \pm 0.8.10^{-2}$ vs $7.1 \pm 0.5.10^{-2}$, $P < 0.05$) and in presence of AngII ($10.6 \pm 1.5.10^{-2}$ vs $7.1 \pm 0.5.10^{-2}$, $P < 0.05$).

2 vs 5.6 ± 0.4 . 10^{-2} , $P < 0.01$). Similarly, expression of CD4 and CD8 in cardiac tissue was lower in LS-fed rats with or without AngII infusion. No significant changes were detected for CD20. Expression of NF- κ Bp65 was reduced in rats fed the low sodium diet in absence or presence of AngII infusion.

Conclusion: Reduction of sodium consumption has a beneficial effect on cardiac morphology in insulin resistance alone or associated with a clear arterial hypertension. This beneficial effect is not related to improvement of insulin resistance. Beside a reduction in the level of hypertension, sodium deprivation seems to act through the blunting of local cardiac inflammation response and the reduction of immune cells (not B cells) infiltration in the heart.

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HDL and signals of endothelial inflammation in type 2 diabetes mellitus

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Background and aims: In type 2 diabetes (T2D), structural as well as compositional changes of HDL have been observed. HDL usually attenuates inflammation, but can under certain conditions become pro-inflammatory. We hypothesized that pro-inflammatory HDL contributes to vascular complications in T2D. In an ongoing study, we compare the effects of HDL from T2D patients on endothelial signals of different human endothelial cells. Here, we report our preliminary results with retinal endothelial cells (REC).

Materials and methods: Anti-inflammatory properties of HDL isolated with iodixanol Gradient centrifugation were measured as TNF- α induced cytokine production and adhesion molecule expression by human retinal endothelial cells (REC). IL-6 and IL-8 production were measured with ELISA and vascular cell adhesion molecule-1 (VCAM1) expression with fluorescence-activated cell sorting (FACS) using fluorescent antibodies. HDL was isolated from T2D patients with both micro- and macrovascular complications (HDL_{T2D+C}, n=3), T2D patients without complications (HDL_{T2D}, n=3) and healthy control subjects (HDL_{Healthy}, n=3).

Results: Pre-incubation of REC with HDL did not alter basal IL-6 and IL-8 production or VCAM1 expression. TNF- α increased IL-6 production from 31.6ng/ml (SD 5.7, n=3)) to 75.0ng/ml (SD 12.2, n=3). After pre-incubation with HDL, TNF- α -induced IL-6 production decreased significantly ($p < 0.0001$) to 42.7ng/ml (SD 7.7, n=9). TNF- α increased IL-8 production from 245.1ng/ml (SD 10.8; n3) to 3618.1ng/ml (SD 728.1; n=3). When pre-incubated with HDL, TNF- α stimulated IL-8 production decreased significantly ($p < 0.0001$) to 2676.1ng/ml (SD 474.0; n=9). The median fluorescent intensity (MFI) of VCAM1-expression on REC was 604.0 units (range 594.2–642.1; n=3) and increased to 7612.8 (range 7331.0–7894.6; n=2) after TNF- α stimulation. Pre-incubation with HDL decreased TNF- α -induced VCAM1 expression significantly ($p < 0.0001$) to 3414.1 units (range 2203.9–4246.2; n=9). The groups were too small for subgroup analyses and comparisons.

Conclusion: We were able to observe inhibitory effects of HDL on TNF- α induced VCAM-1 expression and IL-6 and IL-8 production by Retinal Endothelial Cells. Our ongoing recruitment and testing will increase the power of the study and enable comparisons between the subgroups.

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Soluble dipeptidyl peptidase 4 induces inflammation and proliferation of human vascular smooth muscle cells via protease-activated receptor 2

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Background and aims: Dipeptidyl peptidase 4 (DPP4) is a ubiquitously expressed cell-surface protease and a novel adipokine, as shown by us. Due to DPP4-mediated degradation of the incretin hormone glucagon-like peptide (GLP)-1, inhibition of DPP4 is now widely used as a therapeutic approach for type 2 diabetes mellitus treatment. In addition, DPP4 inhibitors display beneficial cardiovascular effects that are not only GLP-1 mediated. However, nothing is known about direct cellular effects of soluble DPP4 (sDPP4), the circulating form of this enzyme. Previously, we showed that sDPP4 directly induces inflammation and proliferation in human vascular smooth muscle

cells (hVSMC). The aim of the present study was to identify a responsible receptor for the sDPP4-induced effects in hVSMC.

Materials and methods: Primary hVSMC from three distinct donors were exposed to protease-activated receptor 2 (PAR2) specific siRNA or the antagonist GB83. Cells were then treated with sDPP4 for 6 and 24h and the impact of PAR2 on the sDPP4-induced effects was investigated. The sDPP4-induced signaling pathways were assessed by Western blot analysis. To analyze the expression of different cytokines mRNA were measured by real-time PCR. Effects on proliferation were detected by ELISA.

Results: Bioinformatic analysis and signaling signature induced by sDPP4 suggest that sDPP4 might be an agonist for PAR2. Indeed, we found a potential PAR2 binding site in the cysteine-rich region of DPP4 responsible for partner binding. When we aligned the potential PAR2 binding site with the sequence of the crystallized DPP4, we could locate it on the surface of DPP4. Interestingly, PAR2 protein level was significantly higher in hVSMC compared to endothelial cell, adipocytes or skeletal muscle cells. After silencing of PAR2, the sDPP4-induced ERK activation (2.5-fold) as well as the proliferation (1.7-fold) was totally abolished. Additionally, the same effect was observed by the PAR2 antagonist GB83. In accordance to that the sDPP4-induced NF- κ B activation (1.5-fold) as well as the upregulation of the pro-inflammatory cytokines IL-6 and IL-8 (2-fold and 1.9-fold, respectively) could completely be prevented by PAR2 silencing.

Conclusion: In conclusion, in this study we characterized a novel sDPP4-induced signaling cascade in hVSMC. sDPP4 directly and markedly activates the MAPK- and NF- κ B signaling pathway in a PAR2 dependent manner leading to pro-atherogenic changes in hVSMC like increased proliferation and inflammation. Considering the increased circulating levels of sDPP4 in obesity and the upregulation of PAR2 in atherosclerotic lesions, our proposed sDPP4-PAR2 interaction might play an important role in linking obesity to cardiovascular disease.

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Inflammatory stimulation transforms glucose into a deleterious agent in human vascular smooth muscle cells

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Background and aims: Although hyperglycemia is recognized as an independent risk factor for cardiovascular diseases, the links between glucose metabolism and the development of atherosclerosis still require elucidation. We have previously shown that normal vascular cells, which have the capacity to regulate the entry of glucose, are not damaged by high glucose concentration unless they are primed with an inflammatory stimulus such as interleukin (IL)1 β .

Materials and methods: In cultured human vascular smooth muscle cells (VSMC) submitted to normal or high glucose (5.5 or 22 mmol/L), inflammation (1 to 10 ng/mL IL1 β), and/or infection with the glucose transporter GLUT1, the following parameters were analysed: (1) glucose uptake and consumption, as well as lactate production; (2) characterization of GLUT1 by flow cytometry, RT-PCR, and immunofluorescence; (3) determination of inflammatory parameters, such as the expression of inducible nitric oxide synthase (iNOS) and the activity of nuclear factor- κ B (NF- κ B); (4) NADPH oxidase activity; (5) expression of glucose-6-phosphate dehydrogenase (G6PD) and activity of the pentose phosphate pathway (PPP) and; (6) determination of total and reduced glutathione.

Results: In VSMC submitted to high glucose, we show that excess glucose uptake and utilization only occurs in the presence of IL1 β . However, we demonstrate that the simple entry of glucose is not enough to be deleterious in these cells since over-expression of GLUT1 or increased glucose uptake following inhibition of mitochondrial oxidative phosphorylation with sodium azide are not sufficient to trigger inflammatory mechanisms. Our results indicate that, besides allowing glucose entry, IL1 β enhances G6PD expression and activates the PPP in VSMC, thus permitting some of the excess glucose to be metabolized by this route. Such activation provides additional substrate for the enzyme NADPH oxidase and results in increased generation of free radicals. These are necessary intermediates for the inflammatory response since they are required for the activation of iNOS and NF- κ B. The higher the concentration of glucose the more the PPP pathway is activated, giving rise to an increased inflammatory condition which cannot be counterbalanced by

the simultaneous regeneration of reduced glutathione that occurs following activation of the PPP.

Conclusion: A pro-inflammatory stimulus such as IL1 β transforms excess glucose into a deleterious agent in VSMC by causing an increase in glucose uptake and its subsequent diversion into the PPP. This action ultimately promotes the pro-oxidant conditions required for the exaggeration of pro-inflammatory pathways. Interestingly, all these pathways were blunted by the blockade of IL1 receptors with anakinra. These findings can improve our understanding of the mechanisms for the development of diabetic vascular complications and suggest the combination of anti-inflammatory strategies together with glycaemic control for a more efficient prevention of such diseases.

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Nitric oxide hyperproduction and markers of DNA damage in the early phase of diabetic nephropathy in rat streptozotocin diabetes mellitus model

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Background and aims: Reactive oxygen species (ROS) production due to intracellular hyperglycaemia is an important factor that initiates inflammatory pathways leading to diabetic nephropathy. NO hyperproduction in the kidney might lead to nitrosative stress with resulting DNA damage. However, data on NO, nitric oxide synthases (NOS) in the diabetic nephropathy are contradictory. Other enzymes like xanthine oxidoreductase (XOR) might be involved. The aim of this work was to study changes and mechanisms of NO production in the kidneys of rats with streptozotocin (STZ) diabetes mellitus model, along with expression of poly ADP ribose polymerase (PARP) and histone gamma H2AX as markers of DNA damage. In order to modify NO production, we have used 1,4-dihydropyridine (1,4-DHP) class compounds, synthesized in the Latvian Institute of Organic Synthesis.

Materials and methods: Diabetes mellitus in rats was induced by streptozotocin (STZ) 50 mg/kg, i.v. Production of NO in kidneys was monitored by means of ESP spectroscopy of Fe-DETC-NO complex. Different inhibitors of NO synthesis (GdCl₃, aminoguanidine, 1400W, allopurinole) have been applied. 1,4-DHP class drugs have been administered 0,5 mg/kg for 3 days per os. Kidney iNOS, eNOS, XOR, PARP and H2AX mRNA and protein expression were detected by qRT-PCR and immunohistochemistry correspondingly, 12 days after diabetes mellitus induction and after 3 days of treatment with DHP class drugs.

Results: NO production increase in kidneys was stable and potent (from 2.64 \pm 0.97 to 15.04 \pm 2.04 ng/g tissue). Application of non-selective NOS inhibitor aminoguanidine and XOR inhibitor allopurinol resulted in a significant decrease of NO hyperproduction. Further, 1,4-DHP class compounds etafatoron, cerebrocrast and fenofatoron significantly decreased NO production. iNOS and XOR protein expression was upregulated in STZ rat kidney and decreased under etafatoron treatment (iNOS: control 11 \pm 4 cells/mm², STZ: 29 \pm 15 cells/mm², STZ+etafatoron; 13 \pm 6 cells/mm² p<0.05) and (XOR: control 8 \pm 2 cells/mm², STZ 27 \pm 7 cells/mm²; STZ+etafatoron 9 \pm 3 cells/mm², p<0,05.). In kidneys of STZ rats, PARP expression was significantly elevated, and normalized by 1,4-DHP drug AV153. H2AX expression also significantly increased in kidneys of diabetic rats, AV153 did not affect this parameter.

Conclusion: We report increased NO production in kidneys of STZ diabetic rats, along with increased markers of DNA damage, after short duration of severe hyperglycaemia. 1,4-DHP class compounds could normalize increased NO production, iNOS, XOR and PARP hyperexpression in STZ rat kidneys. NO production in the kidney might become a therapeutic target to decrease oxidative stress and progression of diabetic nephropathy.

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Early intervention with GLP-1 analogue prevents memory impairment and tau hyperphosphorylation in diabetic db/db mice

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Background and aims: Recent studies have shown that type 2 diabetes mellitus (T2D) is a risk factor for Alzheimer's disease (AD), and hyperphosphorylated tau protein is a pathophysiological change in hippocampus on the early set of AD, which is called AD associated tau change. Insulin resistance is often associated with T2D and can induce defective insulin signaling in the central nervous system. Glucagon like peptide-1 (GLP-1) stimulates insulin secretion and has been employed in the treatment of T2D. Unlike insulin, GLP-1 and its analogs could cross the brain blood barrier freely. In this study, we investigated the potential effects on AD associated tau protein with treatment with a GLP-1 analog in db/db mice.

Materials and methods: A total of 80 male db/db mice at the age of 2-2.5 weeks were randomly divided into three groups: GLP-1-treated, insulin-treated and control-treated groups. After being acclimatized and received daily subcutaneous saline injections for 1 week, all mice received daily subcutaneous injection of GLP-1 analog, insulin or, as a control, saline right before the dark cycle each day. Five to seven mice from each group were sacrificed every two weeks. Blood, cerebrospinal fluid (CSF) and brain tissues were collected at the second, fourth, sixth and eighth weeks separately after GLP-1 analog or insulin administration for analyses.

Results: We found that GLP-1 analog promoted more beneficial effects on learn memory in db/db mice compared with insulin treatment. The drug reduced hyperphosphorylated tau protein by activating insulin signaling pathway in the hippocampus. On the contrary, insulin peripheral administration had no effects on AD associated tau protein changes. Neither GLP-1 analog nor insulin could stimulate neuronal degeneration.

Conclusion: In summary, our data indicate that early GLP-1 analog intervention improves cognition in db/db mice, an effect likely driven by increasing the activity of insulin signaling pathway in the T2D brain, which leads to the reduction of AD associated tau hyperphosphorylation.

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1323

Effect of hyperglycaemia on the expression of aquaporins in diabetic rat testis

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Background and aims: Diabetes mellitus (DM) is one of the greatest public health threats in modern societies. DM is a well-recognized cause of male sexual dysfunction and impairments of male fertility. Sustained hyperglycemic and hypoinsulinemic states is considered as a major cause of sexual, ejaculatory and erectile dysfunction in human population. Water and solute movement across the epithelium of the male reproductive tract is responsible for balancing the luminal environment for spermatogenesis and plays a pivotal role for establishment of male fertility. Aquaporins (AQPs) are a family of integral membrane proteins allowing the transcellular transport, maintaining water homeostasis and is strongly associated with female and male reproductive systems. In the testis, AQPs play an indirect role in maintaining spermatogenesis, while in the efferent ducts and epididymis; provide environment for the transport and maturation of sperm. The main aim of this study is to determine the effect of hyperglycemia on expression of AQPs in testis of diabetic rats.

Materials and methods: Experimental animals were handled according to the University and institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPC-SEA), Ministry of Social Justice and Empowerment, Government of India (BDU/IAEC/2011/28/29.03.2011). Experimental diabetes in rats was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg body weight). Plasma glucose was measured using glucometer. The mRNA expressions of AQPs (1, 7, 8, 9 & 11) in testis from control and diabetic rats were examined by RT-PCR after a time interval of 4 weeks. All statistical analysis was carried out using GraphPad Prism4 software. All data were presented in mean (\pm) standard deviations (SD).

Results: Plasma glucose was significantly higher in diabetic groups than in controls ($P < 0.05$). The RT-PCR detection of the aquaporins (1, 7, 8, 9 & 11) considered in this study is illustrated in Fig. 1. The level of expression of AQP 1 in diabetic testis was similar to that of control groups. The levels of expression of AQPs 7, 8, 9 and 11 in diabetic testis were significantly higher compared to controls ($P < 0.05$).

Conclusion: In summary, this work describes the expression of AQPs 7, 8, 9, & 11 in testis of diabetic rats were altered compared to controls. Alteration of AQPs expression leads to disruption of water homeostasis and affects spermatogenesis. Recent studies shows that AQPs 7, 8 & 11 plays an important role in spermatogenesis and physiological sperm volume regulation. AQP9 plays a specific role in the transport of water and non-charged solutes in Leydig cells. Furthermore, altered expression and regulation of AQPs have already been demonstrated to be the cause of several disorders of the male reproductive system. The full enlightenment of these molecular interactions and mechanisms may point towards possible therapeutic targets to counteract diabetes induced male subfertility/infertility.

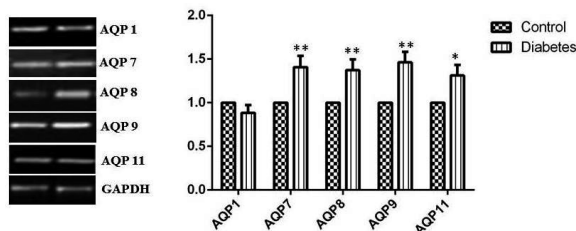


Fig. 1 Expression of AQPs 1, 7, 8, 9, 11 in testis of control and diabetic rat. The gene expression was determined by RT-PCR. Values represent mean \pm S.D (* $P < 0.05$; ** $P < 0.005$)

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Decreased use of experimental animals through optimisation of experimental design for efficacy studies

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Background and aims: Well-designed studies provide the maximum of information from the minimal number of experimental animals. Aim of this study was to explore whether asymmetric experimental designs can reduce number of animals needed without affecting statistical power of the study.

Materials and methods: Data from several efficacy studies in bleomycin-induced lung fibrosis in mice were combined to make tentative estimates of the expected outcomes for newly planned studies. Classically, these types of studies comprise a PBS control group, a bleomycin-induced (bleo) group, and several treatment groups with the same number of animals in all groups. Instead of comparing all groups to each other using ANOVA and post-hoc testing, $k + 1$ planned comparisons were defined in advance: bleo versus PBS control, and bleo versus k treatments (in total $k + 1$ comparisons). Since the bleo group appears in both the comparison with the PBS control and the treatment group, changing the size of this group will have most effect on the statistical power. Since the direction of the effects was anticipated, only one-sided t-tests were used. For fixed type I error, statistical power of the Student t-test was calculated for all combinations of group sizes of 2–30 animals. The optimal asymmetric design achieves the desired statistical power for each planned comparison with the minimum of animals needed.

Results: We calculated that the best asymmetric designs required about 20–25% fewer animals than the best symmetric design.

Conclusion: This demonstrates that substantial reduction of animal use is possible by smarter choices on group size without loss of power. To aid researchers to optimize their experimental design, we developed a freely available sample size calculator (available at www.tno.nl/3R) to compare symmetric and asymmetric designs.

1325

Twice-daily hyperglycaemic spikes accelerate atherosclerotic lesion formation in C57BL/6 mice

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Background and aims: Increasing epidemiological evidence indicates that postprandial hyperglycemia in individuals with impaired glucose tolerance (IGT) is a risk factor for atherosclerotic cardiovascular disease. A number of studies have demonstrated the possible roles of acute glucose fluctuations, a hallmark of IGT, in atherogenesis in cell culture models; e.g., intermittent high glucose exposure-induced oxidative stress, inflammatory response, and subsequent proatherogenic changes in vascular endothelial cells, smooth muscle cells, and macrophages. However, there is little convincing experimental evidence that repetitive glucose fluctuations can actually result in accelerated atherosclerosis in vivo. Hence, the in vivo mechanism underlying IGT-induced atherogenesis is not yet fully understood. In this study, we demonstrated the effect of long-term repetitive hyperglycemic spikes on atherosclerotic lesion formation in mice.

Materials and methods: Female C57BL/6 mice were fed an atherogenic diet (AD; 1.25% w/w cholesterol, 0.5% w/w sodium cholate, 36% energy fat) from 8 to 28 weeks of age. During the 20-week AD feeding period, the mice received 20% glucose solution (50 mg glucose/mouse) (G) or distilled water (W) twice daily by oral gavage (6 days a week). The post-challenge blood glucose changes were monitored on a day in the week 2, 11, and 20. OGTT and insulin tolerance test were performed in the week 19 and 20, respectively. After the AD feeding, serial frozen sections (10 μ m thickness, spanned 450 μ m) of aortic sinus were prepared and the atherosclerotic lesion size was quantitated from the oil red O-stained area (mean of 9 sections, each separated by 50 μ m). Gene expression levels in the thoracic aorta and the plasma

lipid profiles were also analyzed. Data are expressed as mean±SEM (n=8). Differences between G and W were assessed by Student's *t* test.

Results: Blood glucose levels in G increased by ~5 mmol/l above W at 20 min after the glucose ingestions and returned to similar levels to W within 60 min. There are no significant differences in body weight, plasma lipid profiles (total cholesterol, HDL-cholesterol, non-HDL-cholesterol, triacylglycerols, and NEFA), and blood glucose levels in OGTT and insulin tolerance test between G and W. Aortic sinus atherosclerotic lesion size in G was 3.8-fold greater than that of W (22.2 ± 5.7 vs $5.8 \pm 1.8 \times 10^3 \mu\text{m}^2$, $p=0.025$). Gene expression levels of an adhesion molecule *Icam-1* (1.26-fold, $p=0.036$) and a macrophage marker *Cd68* (1.27-fold, $p=0.036$) in thoracic aorta were significantly higher in G relative to those in W.

Conclusion: These results demonstrate that repetitive hyperglycemic spikes can accelerate atherosclerotic lesion formation in vivo independent of basal blood glucose levels, insulin resistance, or plasma lipid profiles, possibly via proatherogenic changes in arterial wall. The repetitive glucose ingestion in mice on AD would be a simple and useful method for studying the pathogenesis, prevention, and treatment of IGT-induced atherosclerotic vascular disorders.

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Cardiac magnetic resonance imaging evaluation after experimental myocardial infarction in streptozotocine induced diabetic rats with high glycaemic variability

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Background and aims: During acute myocardial infarction (MI) in type 2 diabetic (T2D) patients, both hyperglycemia and hypoglycemia (at admission and/or during the hospital stay) are associated with poor outcome. In addition, the impact of glycemic variability in these patients is also discussed: several in vitro and in vivo studies have suggested a deleterious effect of glycemic variability on endothelial function through an increase in oxidative stress. However, there is actually no data concerning the direct impact of this glucose metric on MI morbidity. We address here this question in a rodent diabetic model.

Materials and methods: Twenty-six adult male Wistar rats (315 ± 41 g), synchronized to a reverse 12 hours dark-light cycle were divided in three groups : control (C) (n=7), sham (S) (n=5) and diabetes (D) (n=14). Rats from group D received 70 mg/kg of intraperitoneal (IP) streptozotocin (STZ). Rats from group C and S received vehicle only 70 mg/Kg IP. Group D was divided in two subgroups : diabetes hyperglycemia (Dh) (n=6) and diabetes variability (Dv) (n=8). Glargine was introduced 48 hours after STZ injection : 5 UI/kg/j subcutaneously (SC) at 9:00 AM for Dh rats and 15 UI/kg/j SC at 9:00 AM for Dv rats. Blood glucose monitoring was performed to confirm that glycemia was maintained above 250 mg/dl in Dh sub-group and presented swings below 150 and above 250 mg/dl in Dv sub-group. After 4 weeks, all rats (except in group S) were subjected to ischemia (30 min of transient coronary artery ligation) followed by reperfusion. Cardiac magnetic resonance imaging (CMR) was performed 1 and 3 weeks (w1 and w3) after surgery to assess left ventricular function [end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (LVEF), wall motion score (WMS)] and myocardial edema.

Results: Mean blood glucose was 140 ± 24 , 130 ± 28 and 265 ± 88 mg/dl in group C, S and D (global $p < 0.001$). Glycemic standard deviation, a glycemic variability index, was higher in Dv compared to Dh group (205 vs. 94 mg/dl; $p=0.021$). LVEF (%) at w1/w3 was $47.5 \pm 11.5/46.5 \pm 7.7$; $59.5 \pm 8.5/55.7 \pm 3.7$ and $51.1 \pm 9.3/47.4 \pm 10.7\%$ for group C, S and D, respectively (ns). Likewise, no inter or intra group difference was found for ESV and EDV. However, WMS was increased (indicating abnormal wall motion) after ischemia in group C and D vs S at w1/w3 ($1.40 \pm 0.20/1.35 \pm 0.20$ and $1.56 \pm 0.15/1.50 \pm 0.27$ vs 1 ± 0 ; $p < 0.0001$). Myocardial edema decreased in infarct zone between w1 and w3 in group C (20.3 ± 5.0 vs 14.5 ± 3.2 ; $p < 0.001$) but not in group D (20.4 ± 5.9 vs 21.2 ± 6.3 ; ns). In subgroup analysis, myocardial edema also decreased in infarct zone between w1 and w3 in group Dh (20.6 ± 5.7 vs 18.6 ± 4.1 ; $p < 0.05$) but not in group Dv (20.3 ± 6.0 vs 22.4 ± 6.8 ; ns). In the remote territory, myocardial edema decreased in group Dh but increased in group Dv between w1 and w3 (Dh: 19.3 ± 5.5 vs. 16.7 ± 4.1 ; Dv: 17.7 ± 4.8 vs. 18.9 ± 6.1 ; all $p < 0.05$).

Conclusion: Myocardial edema, reflecting myocardial injuries, does not improve over time in diabetic rats compared to control animals after an experimental MI. This effect is due to the sole subgroup with high glycemic

variability. Furthermore, in this particular subgroup, prolonged edema was also observed in the remote territory. These results suggest that glycemic variability might be more deleterious than persistent hyperglycemia after MI.

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Cardioprotective effect of decorin in type 2 diabetes

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Background and aims: Cardiomyopathy in diabetes is the leading cause of increased mortality in diabetes patients. In the present studies, we investigated the effect of decorin (DCN) gene therapy on left ventricular function, cardiac inflammation and fibrosis in high-fat diet (HFD) and streptozotocin (STZ)-induced type 2 diabetes.

Materials and methods: Type 2 diabetes was induced in male Wistar rats by HFD (60% of calories as fat) and injection of low-dose STZ, (20 mg/kg, intravenously). Diabetic rats were divided into (n=6 for each group) the control group, the GFP-treated group) and the DCN-treated group, received intravenous injection of saline solution, recombinant adeno-associated viral (rAAV)-GFP and rAAV-DCN, respectively. We investigated cardiac inflammation, fibrosis, left ventricular (LV) function at 6 months after gene delivery.

Results: rAAV-DCN Treatment attenuated diabetic cardiomyopathy with improved LV function, cardiac inflammation and fibrosis compared with control animals. These protective effects were associated with increased expression of PKC- α and Hsp70, decreased activation state of IGF-IR, pERK, TGF- β 1 and α -SMA and NF- κ B pathways.

Conclusion: Our results show that rAAV-mediated DCN therapy may be beneficial in the treatment of diabetic Cardiomyopathy.

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Hyperglycaemia does not affect restenosis development after percutaneous transluminal angioplasty in a diabetic rabbit model

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Background and aims: It is clear that hyperglycemia aggravates the development of atherosclerosis. However, whether hyperglycemia promotes prevalence of restenosis remains unknown. In the studies, we investigated the effect of hyperglycemia on restenosis.

Materials and methods: Restenosis was evaluated in two sets of diabetic rabbits models: (1) diabetic restenosis (DR) versus non-diabetic restenosis (NDR); and (2) diabetic atherosclerosis (DA) versus non-diabetic atherosclerosis (NDA).

Results: Our study showed no difference in restenosis rates between the diabetic and the non-diabetic rabbits ($92.33 \pm 5.46\%$ versus $89.17 \pm 7.14\%$, $P > 0.05$). However, the prevalence of stenosis was significantly increased in the DA group compared with NDA group ($72.37 \pm 24.55\%$ versus $35.54 \pm 40.97\%$, $P < 0.05$). Similarly, intima-media thickness and cell proliferation were remarkably higher in the DA group than that of the NDA group, while no difference was found between the DR and NDR groups.

Conclusion: Our results provide evidence that hyperglycemia is an independent risk factor for atherosclerosis, but it does not promote restenosis, and different mechanisms are involved in the development of atherosclerosis and restenosis.

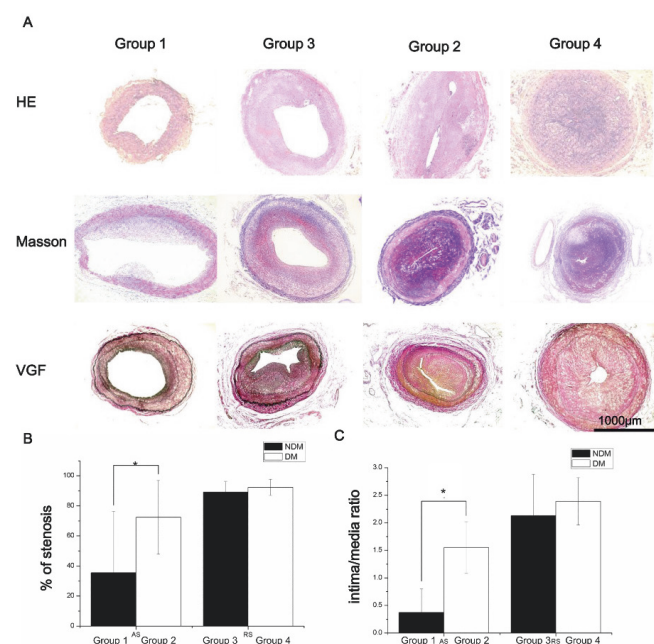


Figure 1 Arteries of rabbits were harvested at day 28 post-surgery. Arteries were plastic embedded and cross sections were stained. A) Cross sections stained with HE, Masson and VGF. B) Stenosis rate of the right iliac artery of rabbits treated with atherosclerotic lesion (group 1, n=6), atherosclerotic lesion+hyperglycemia (group 2, n=4), restenotic lesion (group 3, n=6), restenotic lesion+hyperglycemia (group 4, n=7). C) Intima/Media ratio of the right iliac artery of rabbits treated with atherosclerotic lesion (group 1, n=6), atherosclerotic lesion+hyperglycemia (group 2, n=4), restenotic lesion (group 3, n=6), restenotic lesion+hyperglycemia (group 4, n=7). HE: Hematoxylin and eosin stain, EVG: Elastic Van-Gieson stain, NDM: non-diabetes, DM: diabetes. Magnification 40 \times . A significant ($P<0.05$) from respective control is denoted by ***. Scale bar=1000 μ m.

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Effect of glucose level on the size of myocardial infarction in pigs

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Background and aims: Patients with diabetes and acute myocardial infarction (MI) have a two- to five-fold increased in-hospital mortality compared with non-diabetic patients. Concomitant hypoglycaemia or hyperglycaemia is suggested to worsen the acute prognosis of MI. Mortality after MI is associated with necrotic damage to the myocardium. Consequently, minimising the necrotic area is warranted. Our aim was to investigate the effect of the acute plasma glucose level on the size of MI in a closed-chest pig model.

Materials and methods: 38 non-diabetic Danish/Landrace female pigs were randomised to three different plasma glucose levels: hypoglycaemia (2.1 ± 1.0 mmol/l; 15 pigs (mean \pm SD)); normoglycaemia (5.8 ± 1.6 mmol/l; 12 pigs) and hyperglycaemia (21.5 ± 2.9 mmol/l; 11 pigs). After 30 minutes of steady state with plasma glucose levels within target (1.8–2.2 mmol/l, 5–7 mmol/l, 22–23 mmol/l, respectively) MI was induced. Using a closed-chest model a balloon catheter was inserted via the internal carotid artery to the left anterior descending coronary artery and inflated for 30 minutes. Afterwards the heart was reperfused while the plasma glucose level remained in target for 2 hours. Following sternum split an in-vivo staining with Evans Blue was performed. The heart was subsequently taken out and in-vitro stained with TTC to delineate the necrotic tissue from the viable tissue (area at risk (AAR)).

Results: Twenty-five (66%) pigs developed ventricular fibrillation (VF) during the reperfusion period. Of these 7 pigs died. There was no difference in incidence of VF ($p=1.0$) or death ($p=0.22$) between the glycaemic groups. No

significant differences in infarction size, AAR or infarction/AAR ratio were detectable between the groups.

Conclusion: In a closed-chest pig model the acute glucose level did neither affect cardiac morbidity or mortality nor the size of the myocardial infarction.

Table 1.

	VF (N)	Mortality (N)	Infarction (mm ²) (mean \pm SD)	AAR (mm ²) (mean \pm SD)	Infarct/AAR ratio (mean \pm SD)
Hypoglycaemia (15 pigs)	10	5	201 \pm 135	617 \pm 180	0.36 \pm 0.24
Normoglycaemia (12 pigs)	8	1	154 \pm 139	633 \pm 206	0.25 \pm 0.23
Hyperglycaemia (11 pigs)	7	1	134 \pm 103	618 \pm 327	0.31 \pm 0.30
p-value	1.0	0.22	0.60	0.97	0.60

Supported by: Research Foundation of Nordsjællands Hospital, Hillerød, Denmark

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Dipeptidyl peptidase-4 inhibition in a rat model of ischaemia-reperfusion injury may accelerate tubular regeneration but does not improve glomerular filtration rate

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Background and aims: Dipeptidyl peptidase (DPP)-4 inhibitors have been shown to have protective effects on ischaemia-reperfusion injury (IRI) of the heart and lung. As the DPP-4 enzyme and its substrates are also expressed in the kidney, we studied the effects of the DPP-4 inhibitors linagliptin, vildagliptin, and sitagliptin on the outcome of IR-induced acute kidney injury in uninephrectomised rats.

Materials and methods: Male Wistar rats obtained from Charles River (Germany) weighing 150–170 g were subjected to uninephrectomy. Two weeks later, the remaining kidney was exposed to IRI by clamping the renal artery for 30 min; sham surgery was performed without clamping. The rats (n=10–14 per group) received DPP-4 inhibitor treatment once-daily with either linagliptin (1.5 mg/kg/day), vildagliptin (8 mg/kg/day), sitagliptin (30 mg/kg/day), or vehicle via gavage on the 3 consecutive days prior to IRI. An additional group was treated with sitagliptin until study end in order to inhibit DPP-4 activity during the entire experiment. This group was treated initially with sitagliptin 30 mg/kg/day; after induction of IRI, the dose was adjusted to 15 mg/kg/day because of renal failure. Levels of plasma cystatin C (a biomarker of glomerular filtration rate) and clusterin (a biomarker of tubular regeneration) were measured at 0, 24, 48, 72, and 168 hours after IRI or sham operation.

Results: Levels of active GLP-1 increased 3–4 fold in all DPP-4 inhibitor treatment groups versus the placebo group, up to 24 hours after clamping ($p<0.05$ for all comparisons). Levels of plasma cystatin C peaked 48 hours after clamping and increased from 1454 ± 320 ng/ml in the sham-operated group to 1728 ± 323 ng/ml in the placebo-treated group with IRI ($p=0.059$). This increase was unaffected by treatment with either linagliptin, vildagliptin, or sitagliptin. Similarly, the area under the curve for plasma cystatin C (0 to 168 hours after IRI) was comparable across all treatment groups. At study end, levels of plasma clusterin were lowest in the placebo-treated group (97.14 ± 15.71 μ g/ml) and significantly higher in all treatment groups (linagliptin: 116.53 ± 25.28 μ g/ml; vildagliptin: 111.28 ± 17.78 μ g/ml; sitagliptin: 113.53 ± 16.45 μ g/ml; extended sitagliptin treatment: 114.99 ± 16.00 μ g/ml; $p<0.05$ for all comparisons).

Conclusion: In rats with acute IRI of the kidney, DPP-4 inhibition does not alter the glomerular filtration rate but may enhance tubular regeneration. These findings were observed when DPP-4 activity was inhibited prior to IRI and for one week following IRI.

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Morphometric characteristics of the air-blood barrier in experimental diabetes mellitusO. Pivovarova¹, E. Rozova², B. Mankovsky³;¹State Establishment „Lugansk State Medical University, Lugansk,²Bogomoletz Institute of Physiology, Kyiv, ³P.L. Shupyk National Medical Academy of Post-Graduate Education, Kyiv, Ukraine.

Background and aims: Respiratory diseases contribute significantly to mortality and morbidity in patients with diabetes mellitus. However, the mechanisms underlying the lung damage in diabetic patients are not fully understood. The air-blood barrier (ABB) performs an important role in functioning of the respiratory system but its structure in diabetes was not studied in detail. Therefore, the aim of this study was to investigate specific histological features of the ABB in the streptozotocin (STZ)-induced diabetes.

Materials and methods: The study was performed on 47 STZ-induced diabetic and 43 control male Wistar rats. Diabetes was induced by single intraperitoneal injection of STZ (SIGMA, USA) 60 mg/kg in 0.1 M citrate buffer, pH 4.5. The tissue for the further evaluation was collected after 18 weeks of diabetes. The diabetic rats were not treated with insulin. The morphometric assessment was performed using the electron microscope PEM-125K and the data were analyzed using the software Image Tool Version 3 (USA). The thickness of arithmetic average (τ) and harmonic average (τ_h) were calculated.

Results: We found the significant increase of thicknesses of τ and τ_h of ABB in STZ-induced diabetic rats compared to control group - τ were $302,7 \pm 11,4$ nm and $163,4 \pm 6,8$ nm, τ_h were - $289,5 \pm 12,5$ nm and $155,6 \pm 5,4$ nm, respectively, in diabetic and control animals, $p < 0,01$. These changes could indicate the hyperhydration. Also, τ and τ_h of epithelial layer of ABB in the STZ-induced diabetic rats were significantly elevated ($98,6 \pm 2,2$ nm and $91,5 \pm 1,7$ nm, respectively), compared to controls ($71,6 \pm 3,5$ nm and $65,1 \pm 2,1$ nm), $p < 0,05$. The values of τ , τ_h of the interstitium of ABB in the STZ-induced diabetic rats were significantly higher than in non-diabetic animals - $92,4 \pm 4,3$ nm, $97,6 \pm 5,2$ nm in diabetes and $49,2 \pm 3,1$ nm and $46,1 \pm 3,3$ nm, in controls, respectively, $p < 0,001$. In the STZ-induced diabetic rats, τ and τ_h of endothelium of ABB were $118,8 \pm 7,1$ nm and $121,7 \pm 4,2$ nm, whereas in the controls there were $63,9 \pm 3,3$ nm and $50,1 \pm 3,2$ nm respectively, $p < 0,001$.

Conclusion: We found the significant thickening of all layers of the ABB, including epithelial, which may underlie the development of intraalveolar edema in the STZ-induced diabetic rats. Permeability changes caused by hyperhydration of ABB can lead to the disturbance of the oxygen transport and the development of hypoxia in diabetic rats.

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Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatoesteatosis and cardiovascular complicationsC. Vernochet¹, F. Damilano¹, A. Mourier², O. Bezy¹, M.A. Mori¹, G. Smith¹, A. Rosenzweig³, N.-G. Larsson², R.C. Kahn¹;¹Joslin Diabetes Center, ²Department of Mitochondrial Biology, Max Planck Institute for Biology of Ageing, ³Cardiovascular Division of the Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA.

Background and aims: Mitochondrial dysfunction in adipose tissue occurs in obesity, type 2 diabetes and some forms of lipodystrophy, but whether this dysfunction contributes to or is the result of these disorders is unknown.

Materials and methods: To investigate the physiological consequences of severe mitochondrial impairment in adipose tissue, we generated mice deficient for the mitochondrial transcription factor TFAM in adipocytes by using mice carrying Adiponectin-Cre and TFAM floxed alleles.

Results: These Adipo-TFAM KO mice had a 75-81% reduction of TFAM in subcutaneous and intra-abdominal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT), and this resulted in decreased expression and enzymatic activity of proteins in complexes I, III, and IV of the electron transport chain (ETC). This mitochondrial dysfunction led to adipocyte death and inflammation in WAT and a “whitening” of BAT. As a result, Adipo-TFAM KO mice were resistant to weight gain, but exhibited insulin resistance, on both normal chow and high fat diets.

Conclusion: These lipodystrophic mice also developed hypertension, cardiac hypertrophy and cardiac dysfunction. Thus, isolated mitochondrial dysfunction in adipose tissue can lead a syndrome of lipodystrophy with metabolic syndrome and cardiovascular complications

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